



Optimizer

for Mobility-Shift Assays

P/N 111917, Rev. 03

User Guide

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1 Introduction

The *Optimizer* for mobility-shift assays is used to systematically determine the optimal separation conditions (of voltage, pressure, and buffer sip time) for a Mobility-Shift Assay. The *Optimizer* uses a mathematical model of the electrokinetic and pressure driven transport in the LabChip microfluidic devices to predict the resolution of product and substrate peaks for any applied voltage and pressure.

You will use the follow steps to determine the best separation conditions:

1. Install the *Optimizer* spreadsheet.
2. Open the *Optimizer_Template* Excel workbook.
3. Enter the amino acid sequence of your peptides.
4. Generate the product peptide.
5. Perform the parameter measuring experiment (to measure the peptide mobility, electroosmotic flow, and buffer viscosity).
6. Use the *Optimizer* spreadsheet to determine the best separation conditions (pressure, voltage, and sip times) required to achieve your desired throughput, data quality, and unattended run time.
7. Test the separation conditions.

Each step is described in more detail in the following section.

2 Step-By-Step Guide to Using the *Optimizer* Spreadsheet

2.1 *Install the Program*

To use the *Optimizer* Spreadsheet, you must have a computer with Windows XP, Windows Vista, or Windows 7, and Microsoft Excel 2003 or higher (Security Level: Medium or Low) installed. Install the *Optimizer* Spreadsheet by running "Setup.exe" from the installation disk. Most of the program resides in a Microsoft Excel Add-in module that will be loaded and configured by the installer. See the *Optimizer* Release Notes for more details.

2.2 *Open the Optimizer_Template*

On the Windows Start menu, select **Programs**→**Caliper Life Sciences**→**Optimizer**. Microsoft Excel starts and a new workbook is created based on the *Optimizer_Template*.

It is best to start with a new instance of the template workbook for each new assay and/or measurement, rather than reusing a workbook that has been used before. It is especially important to avoid reusing a workbook that has been modified.

2.3 *Enter the Amino Acid Sequence of your Peptide*

In the Microsoft Excel spreadsheet, select the *A Priori Estimates* worksheet and describe the substrate and product peptides by entering their amino acid sequence and termination method in the appropriate cells (see Figure 1). To enter the sequence, use the single letter abbreviation for each amino acid. To enter a phosphate, enclose PO₃ in parentheses, (PO₃), as shown in the illustration in Figure 1.

Inputs:		Outputs:							
Substrate Amino Acid Sequence				Product Amino Acid Sequence				Buffer pH	Temperature (C)
(5FAM)LRRASLG(CONH2)				(5FAM)LRRAS(po3)LG(CONH2)				7.3	27
Substrate Molecular Weight (g/mol)	Substrate Charge	Product Molecular Weight (g/mol)	Product Charge	Buffer Viscosity (cP)	Sub Mobility (cm/V*s)	Prod Mobility (cm/V*s)	Sub Diffusivity (cm ² /s)	Prod Diffusivity (cm ² /s)	
1130	0.11	1209	-1.32	1.35	5.572E-06	-6.258E-05	1.172E-06	1.146E-06	
Recommended Measurement Job File = 'xxxxx_Neg_MobilityViscosityEstimate.Job'									
Where xxxxx is the chip number, for example, EZ Reader 4-Sipper or EZ Reader 12-Sipper									

Figure 1. The *A Priori Estimate* worksheet. The substrate and product peptides must be described by entering their amino acid sequence and termination into the appropriate cells on this sheet.

This worksheet calculates a molecular diffusivity estimate that is used by other worksheets to predict assay performance. The output values for the electrophoretic mobility should be treated as “ballpark” estimates based simply on the mass and charge of the molecules. They are displayed for your information only - for best results, the electrophoretic mobility must be measured in the assay buffer that you intend to use for screening. Use the “Recommended Measurement Job File” to measure the mobility, as describe in the next section.

2.4 Generate the Peptide Product

Purchase a phosphorylated peptide, or use an enzymatic reaction to generate pure product peptide. About 70% product is sufficient for reliable measurement - measurement errors may result if the product is contaminated by more than about 30% with the substrate peptide.

2.5 Perform the Viscosity/Mobility Measurement Experiment

It is important to perform the viscosity/mobility measurement experiment using your particular assay buffer. Caliper’s recommended buffer (Caliper 760367-P) is described below as a guide. If your assay requires any changes to the buffer, be sure to make the changes when performing the viscosity/mobility measurement.

Materials needed:

- ❑ 400 mL of 1 X Separation Buffer with DMSO, for example:
 - 100 mM HEPES, pH 7.3
 - 0.015% Brij-35
 - 5.0% DMSO
 - 0.1% Coating Reagent 3
 - 1 mM EDTA
 - 2mL of 10uM CR-8 (Caliper 760278)
 - Note: it is important to accurately match the composition of the separation buffer and the buffer in the chip and the buffer used for the samples on the plate.
- ❑ ~ 1 to 2 uL of substrate stock solution (>500 uM).
- ❑ Electrically neutral dye. For example, Rhodamine B (Sigma 25,242-5), or Bodipy Fluorescein Arginine (included in Caliper LabChip Test Kit, PN 760403).
- ❑ Product solution prepared above (Section 1.4).
- ❑ Caliper Mobility-Shift assay chip (EZ Reader 4- or 12-sipper) at room temperature.

Procedure:

1. Prepare approximately 1 uM solutions of the substrate, product, and neutral dye. Also prepare a mixture of substrate and product at about 1 uM each. If using an EZ Reader 4-sipper chip, make 300 uL of each solution. If using an EZ Reader 12-sipper chip, make 1.2 mL of each solution.

2. Plate Preparation:
 Prepare a Greiner 384 well microtiter plate as illustrated in Figure 2 for use with 4-sipper chips or Figure 3 for use with 12-sipper chips, where:
 - N = 70 μ l of neutral dye (1 μ M Bodipy Fluorescein Arginine, or 150 μ M Rhodamine B).
 - Sub = 70 μ l of 1 μ M substrate peptide, in separation buffer.
 - Prod = 70 μ l of 1 μ M product peptide, in separation buffer.
 S/P = Substrate/Product mixture: 70 μ L of 1 μ M product and 1 μ M substrate.
 (The S/P wells will be sipped to test the separation using the "Make an Experiment/Job File" function (see Section 1.7)).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	N	Sub	Prod	S/P	N	Sub	Prod	S/P																
B																								
C																								
D																								
E	N	Sub	Prod	S/P	N	Sub	Prod	S/P																
F																								

Figure 2. Plate layout for use with 4-sipper chips.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	N	N	N	N	N	N	Sub	Sub	Sub	Sub	Sub	Sub	Prod	Prod	Prod	Prod	Prod	Prod	S/P	S/P	S/P	S/P	S/P	S/P
B																								
C																								
D																								
E	N	N	N	N	N	N	Sub	Sub	Sub	Sub	Sub	Sub	Prod	Prod	Prod	Prod	Prod	Prod	S/P	S/P	S/P	S/P	S/P	S/P
F																								

Figure 3. Plate layout for use with 12-sipper chips.

3. Prepare the instrument and refresh the separation buffer if needed.
4. Prepare the chip, and load it into the instrument.
5. Place the plate into the instrument.
6. Run the appropriate measurement job file for your chip and peptide:
 - The *Optimizer* installation CD contains several job files to be used for the measurement experiment:

Chip	Peptide Charge	Job Filename
EZReader 4	Positive	EZReader4-sipper_Pos_ViscosityMobilityEstimate
EZReader 4	Negative	EZReader4-sipper_Neg_ViscosityMobilityEstimate
EZReader 12	Positive	EZReader12-sipper_Pos_ViscosityMobilityEstimate
EZReader 12	Negative	EZReader12-sipper_Neg_ViscosityMobilityEstimate

The spreadsheet makes a recommendation as to which job file will make the best measurement of your peptide (after you type in the sequence of your peptide) on the *A Priori Estimates* worksheet (see Figure 1).

7. The experiment consists of a series of sample sips at varying pressure and voltage conditions. You should see 9 peaks in each data trace. The measurement job file takes about 18 minutes to run through to completion.

2.6 Analyze the Data

1. Working in the *Optimizer* spreadsheet, open the *Experimental Estimates* worksheet (see Figure 4).
2. Click the [Run Analysis Macro] button (see Figure 4), and then enter the name of the data (.rda) file that was created in Step 1.5.11. The analysis macro will fill in all of the input cells on this worksheet, so you do not need to enter any data other than the file name.
3. Review the data plot for each sipper in the *Peak Finder Results* worksheet (shown in Figure 5). Check to be sure that the peak finder found all of the peaks correctly. If you do not see a yellow marker on each of the nine prominent peaks, read section 2.1 to learn how you can correct the problem.
4. Once you are satisfied that the correct peak arrival times are in the table, click the [Accept] button to complete the analysis.
5. The active window will switch back to the *Experimental Estimates* worksheet after the [Accept] button is clicked so you can see the results, but there is nothing further to do on that sheet except view the results.

Run Analysis Macro		Auto Mode		Manual Mode		Experiment / Job Properties:										Results Output:													
Filename	Chip	Applied Pressure (psi)	Measure Voltage1 (V)	Measure Voltage2 (V)	Initial Delay (s)	Buffer Dwell (s)	Sip Time (s)	Robot Movement Time (s)	Startup time (s)																				
C:\Projects\EZ Reader	EZ Reader 4-Sipper	-1.5	-1400	1400	30	106	0.2	1.6	32.5																				
Trial (channel)	Peak Arrival with: Voltage Off			...1st Voltage Applied			...2nd Voltage Applied																						
	Neutral Peak Time (s)	Substrate Peak Time (s)	Product Peak Time (s)	Neutral Peak Time (s)	Substrate Peak Time (s)	Product Peak Time (s)	Neutral Peak Time (s)	Substrate Peak Time (s)	Product Peak Time (s)																				
1	116.40	225.80	335.10	443.90	553.50	651.80	771.40	880.30	1013.90																				
2	117.40	226.80	336.00	444.80	554.40	652.40	772.30	881.20	1015.80																				
3	116.60	226.10	335.20	444.20	553.80	652.00	771.70	880.60	1014.70																				
4	116.40	225.90	334.90	443.90	553.60	651.70	771.50	880.30	1014.20																				
5																													
6																													
7																													
8																													
9																													
10																													
11																													
12																													
Mean	116.70	226.15	335.30	444.20	553.82	651.98	771.73	880.60	1014.65																				
Sipper-to-Detector Time (s):										Electro-Mobilities (cm ² /V*s)																			
1	83.90	84.10	84.20	83.80	84.20	73.30	83.70	83.40	107.80	2.72E-06	2.09E-06	-1.02E-04	-1.00E-04	-6.87E-07	1.38E-06	1.38	1.00												
2	84.90	85.10	85.10	84.70	85.10	73.90	84.60	84.30	109.70	2.68E-06	2.06E-06	-1.03E-04	-1.01E-04	-1.35E-06	2.02E-06	1.39	1.00												
3	84.10	84.40	84.30	84.10	84.50	73.50	84.00	83.70	108.60	2.70E-06	2.07E-06	-1.02E-04	-1.01E-04	9.36E-11	6.83E-07	1.38	1.00												
4	83.90	84.20	84.00	83.80	84.30	73.20	83.80	83.40	108.10	3.40E-06	2.79E-06	-1.03E-04	-1.00E-04	-6.87E-07	6.87E-07	1.38	1.00												
5																													
6																													
7																													
8																													
9																													
10																													
11																													
12																													
Mean	84.20	84.45	84.40	84.10	84.52	73.48	84.03	83.70	108.55	2.87E-06	2.25E-06	-1.02E-04	-1.01E-04	-6.80E-07	1.19E-06	1.38	1.00												
Estimated Viscosity (cP)	Substrate Mobility (cm ² /V*s)	Product Mobility (cm ² /V*s)	EO Mobility (cm ² /V*s)	Electrical Resistance (MΩ)	Substrate Retention Factor	Product Retention Factor	Mean Current (μA)	Observed Current1 (μA)	Observed Current2 (μA)	Observed Current3 (μA)	Observed Current4 (μA)	Observed Current5 (μA)	Observed Current6 (μA)																
1.38	2.56E-06	-1.02E-04	2.56E-07	201.03	1.00	1.00	6.96	6.94	6.99																				
Summary Electro-Mobilities (cm ² /V*s)																													
1st V	Sub μEp	Prod μEp	μEo																										
2nd V	2.87E-06	-1.02E-04	-6.80E-07																										
Mean	2.25E-06	-1.01E-04	1.19E-06																										
Difference	6.211E-07	1.628E-06	1.872E-06																										

Figure 4. The *Experimental Estimates* worksheet. Use this worksheet to analyze the data generated by the Viscosity/Mobility measurement experiment.

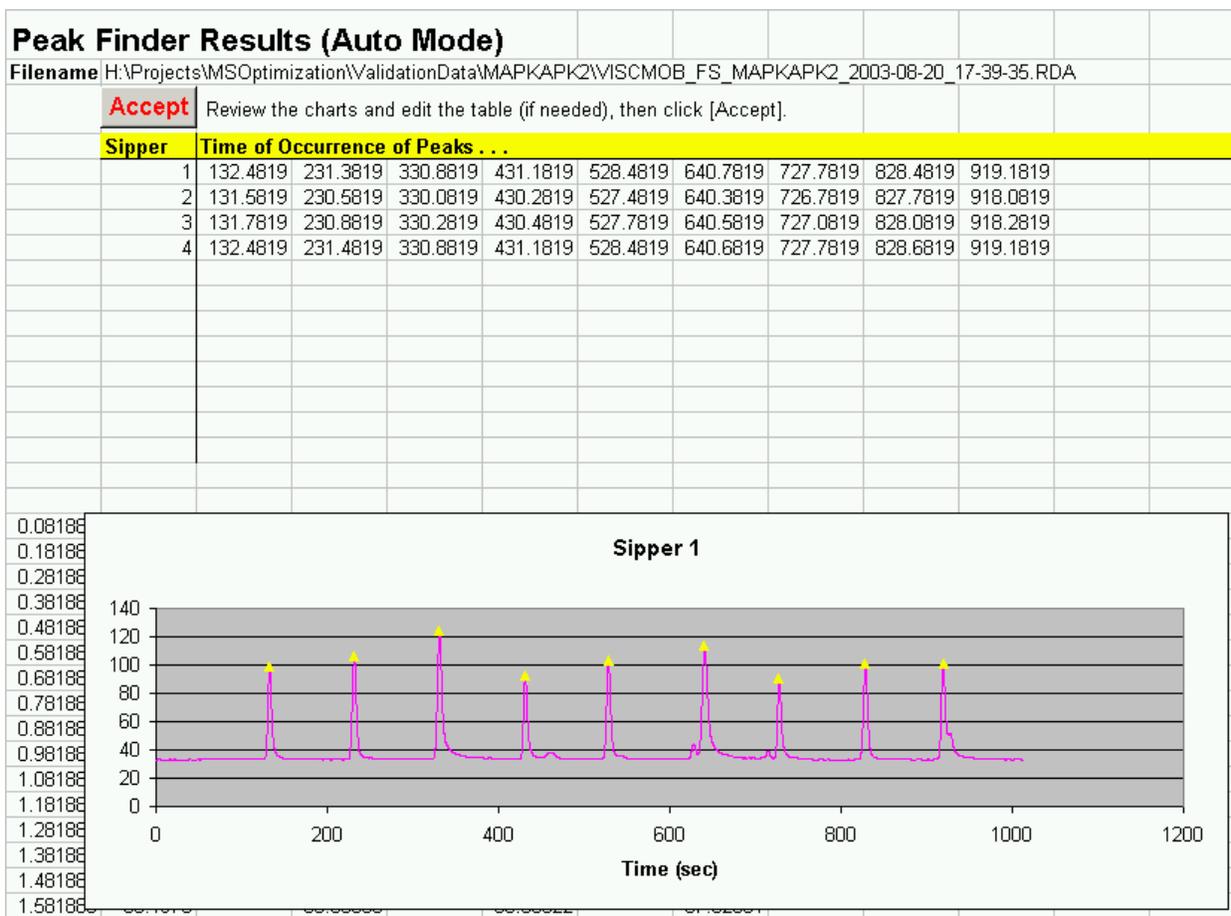


Figure 5. The *Peak Finder Results* worksheet that shows the data generated by the Viscosity/Mobility measurement experiment. Be sure to click the [Accept] button to enter the peak arrival times into the program.

2.7 Select the Best Operating Parameters

1. Switch to the *Experimental Assay Predictions* worksheet (see Figure 6). The input cells on this sheet are all filled in by the analysis macro that was run in the previous step, so you do not need to enter any values.
2. Select the set of operating parameters that best meet your needs of throughput and unattended run time. Consider the columns labeled “Buffer Lifetime (hr)” and “Time for One Plate (min)”. Select the set of parameters that will give adequate Buffer Lifetime without taking too long to run each plate. You can select any set of parameters from the table, but a good choice is to select the conditions that will give a Buffer Lifetime of about 36 hours, using a pressure in the range -1.2 to -2 psi and an applied voltage lower than 2500V (positive or negative). To make your selection, click on one of the Radio Buttons to the left of the table—your selection will be highlighted in blue. (If there are no valid conditions available in the table (for example, all ‘Impossible’) or if the available conditions do not meet your needs for throughput or buffer lifetime, read the troubleshooting sections 2.1 and 2.3 or call Caliper Customer Service for help.)
3. Click [Make an Experiment/Job File] and enter a file name in the dialog box. The spreadsheet will create an Experiment or Job file with all of the parameters (e.g., Voltage, Pressure, sipping time, etc.) set appropriately to achieve good performance for this particular assay.

Off-Chip Mobility-Shift Assay Voltage/Pressure Predictions									
Use this page to predict Voltage/Pressure pairs that will produce your desired resolution. This worksheet takes its input from the Experimental Estimates sheet. Use this sheet for best results when you have measured the peptide mobility.									
Version = 3.3.28.0; 3.2.0; OffChip									
Inputs:									
Chip	Viscosity (cP)	Sub Mobility (cm/V*s)	Prod Mobility (cm/V*s)	EO Mobility (cm/V*s)	Sub Diffusivity (cm ² /s)	Prod Diffusivity (cm ² /s)	Sip Time (s)	Robot Move Time (s)	Sample Sip Time (s)
EZ Reader 4-Sip	1.38	2.56E-06	-1.02E-04	2.56E-07	1.17E-06	1.15E-06	1.8	1.6	0.2
Desired Resolution	Resolution Between Wells	Buffer pH	[Buffer1] (mM)	pK1	[Buffer2] (mM)	pK2	Electrical Resistance (MΩ)	Make an Experiment/Job File	
1.5	1.8	7.3	100	7.55	0		201		
Outputs:									
Pressure (psi)	Applied Voltage (V)	Post Sample	Post Dye Buffer (s)	Final Delay (s)	Time for One Plate	Buffer Lifetime	Peak Order		
<input type="radio"/>	-1	-700	25.9	38.8	145	55.5	118.0	Prod First	
<input type="radio"/>	-1.1	-800	24.0	36.0	133	51.9	103.3	Prod First	
<input type="radio"/>	-1.2	-950	23.4	35.1	123	50.6	87.0	Prod First	
<input type="radio"/>	-1.3	-1050	21.7	32.6	114	47.4	78.7	Prod First	
<input type="radio"/>	-1.4	-1200	21.0	31.5	106	45.9	68.9	Prod First	
<input type="radio"/>	-1.5	-1350	20.2	30.3	100	44.4	61.2	Prod First	
<input type="radio"/>	-1.6	-1500	19.4	29.1	94	42.8	55.1	Prod First	
<input type="radio"/>	-1.7	-1650	18.6	27.9	89	41.2	50.1	Prod First	
<input type="radio"/>	-1.8	-1850	18.2	27.3	85	40.5	44.7	Prod First	
<input type="radio"/>	-1.9	-2000	17.4	26.2	80	39.0	41.3	Prod First	
<input checked="" type="radio"/>	-2	-2200	17.0	25.5	77	38.2	37.6	Prod First	
<input type="radio"/>	-2.1	-2400	16.6	24.8	74	37.3	34.4	Prod First	
<input type="radio"/>	-2.2	-2600	16.1	24.1	71	36.4	31.8	Prod First	
<input type="radio"/>	-2.3	-2800.00	15.61	23.4	68	35.5	29.5	Prod First	
<input type="radio"/>	-1.2	650	28.4	28.4	122	57.5	106.8	Sub First	
<input type="radio"/>	-1.3	750	28.4	28.4	113	57.5	92.5	Sub First	
<input type="radio"/>	-1.4	850	28.3	28.3	105	57.0	81.6	Sub First	
<input type="radio"/>	-1.5	900	25.7	25.7	99	52.4	77.1	Sub First	
<input type="radio"/>	-1.6	1000	25.4	25.4	93	51.9	69.4	Sub First	
<input type="radio"/>	-1.7	1100	25.0	25.0	88	51.1	63.1	Sub First	
<input type="radio"/>	-1.8	1200	24.6	24.6	84	50.3	57.8	Sub First	
<input type="radio"/>	-1.9	1300	24.1	24.1	80	49.4	53.4	Sub First	
<input type="radio"/>	-2	1400	23.6	23.6	76	48.4	49.6	Sub First	
<input type="radio"/>	-2.1	1500	23.1	23.1	73	47.4	46.3	Sub First	
<input type="radio"/>	-2.2	1600	22.5	22.5	70	46.4	43.4	Sub First	
<input type="radio"/>	-2.3	1700	22.0	22.0	67	45.5	40.8	Sub First	
<input type="radio"/>	-2.4	1800	21.5	21.5	65	44.5	38.6	Sub First	
<input type="radio"/>	-2.5	1900	21.0	21.0	62	43.5	36.5	Sub First	

Figure 6. A Typical Mobility-Shift Assay Prediction worksheet. Click the “radio button” at the left to select the set of parameters that will give acceptable throughput (Time for One Plate) and Buffer Lifetime, and then click [Make an Experiment/Job File].

2.8 Test the Separation Conditions

This step uses the mixture of product and substrate that was put on the plate during the measurement experiment setup (“S/P” in Figures 2 and 3), to test the separation conditions.

1. At the Caliper instrument, open the Job or Experiment file that was created in the previous step.
2. Set the Data Location and file naming convention as appropriate for your application, and save the file.
3. With the same plate (previously used for the measurement experiment) on the plate tray, start the run. You can skip the preparation steps in the start wizard (or Setup wizard on EZ Reader instruments) since you have already completed them during the first part of the experiment.
4. Visually inspect the resulting data file to be sure that the “Resolution” (between substrate and product peaks) and “Between Well Resolution” are acceptable (see example in Figure 7).

5. Assuming the Separation Check data is acceptable, you can use the Separation Check file as a template for your screening job or experiment (just modify the Dwell Pattern, Data Location, etc., and save it with a different name). If the Separation Check data file looks unacceptably different than the example in Figure 7, read Section A.2 *Troubleshooting Resolution Errors*.

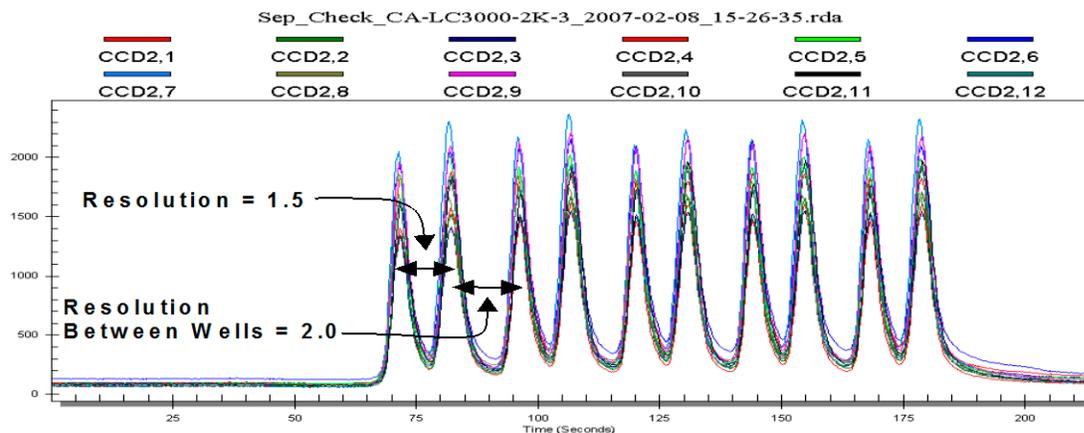


Figure 7. Example of a good Separation Check file, illustrating the two most important characteristics to check: Resolution = 1.5, and Resolution Between Wells = 2.0.

3 More about Measuring the Mobility and Viscosity

To obtain useful predictions from the spreadsheet, it is essential to have good estimates of the following:

- 1) Viscosity of the assay buffer,
- 2) Electrophoretic mobility of the substrate peptide,
- 3) Electrophoretic mobility of the product peptide, and
- 4) Electroosmotic mobility of the chip-buffer system.

The following sections describe the measurement experiment in more detail and offers advice for troubleshooting measurement problem and analyzing some of the more complicated data files that may come about.

The general idea of the experiment is to sip a neutral dye (e.g., BFA, Rhodamine-B), and then sip a sample of substrate, then product with first no voltage applied, then with a negative voltage applied, and finally with a positive voltage applied, as illustrated in Figure 8.

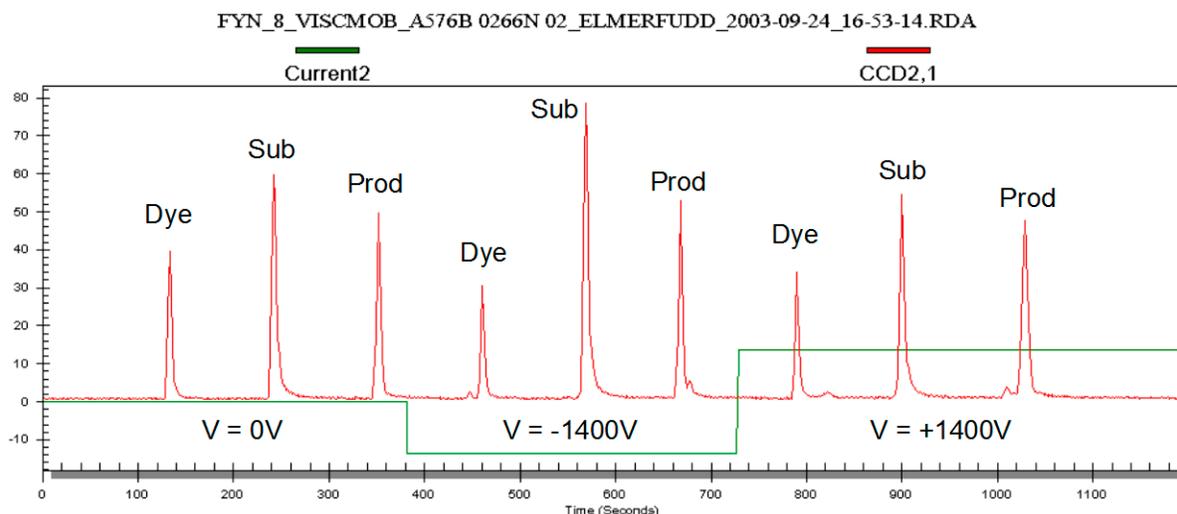


Figure 8. Typical data generated by the Viscosity/Mobility measurement experiment, annotated to clarify the origin of each peak and show the timing of the voltage transitions.

The viscosity of the buffer is measured by using the transit time (from sipper to detector) of the neutral dye, and knowledge of the applied pressure and chip geometry to solve for the viscosity required to produce the observed transit time. The mobilities are similarly estimated by using the neutral dye as a reference and comparing its travel time with the travel time of the substrate and the product while a voltage is applied. Any difference in observed transit time between the neutral dye and substrate or product is assumed to be due to different electrophoretic mobilities of the molecules. Since the electrophoretic mobility of the neutral dye is known to be zero, it is used as a reference to estimate the mobilities of the other molecules.

3.1 Troubleshooting Analysis of the Data

The *Optimizer* spreadsheet contains all the functions and look-up tables needed to analyze the data from the viscosity/mobility measurement experiment. The analysis is performed in two steps. First, the raw data file is processed to extract the time of occurrence of each of the nine peaks shown in Figure 8. Next, the peak arrival times are transferred into the *Experimental Estimates* worksheet, and the calculations of viscosity, mobility, etc. are performed. In most cases, the analysis macro yields results without any intervention from the user. However, sometimes the measurement experiment produces a data file that cannot be analyzed automatically. In this case, the spreadsheet allows the user to override the automatic peak finding by typing in the correct value for the arrival time of one or more of the peaks.

After the automatic peak finder has completed processing the data file, the user is asked to review the plot for each sipper to verify that the nine correct peaks have been found, and then click the [Accept] button if that is true. Most of the time there are only nine prominent peaks in the file and it is obvious that the correct ones have been identified. However, Figure 9 shows an example where a few extra peaks are present (probably due to some contamination of reagents), and one of the extra peaks (labeled “???” in the figure) is more prominent than the neutral dye peak that the analysis program is actually trying to find. In the example, the analysis program has incorrectly identified the “???” Peak as the “dye peak”, and it would make a large measurement error if it were not corrected. Perhaps the best thing to do in a case like this is to identify the source of the supposed contamination, correct that problem, and repeat the measurement experiment with fresh reagents. In the event that some extra peak(s) cannot be removed from the experimental results, the spreadsheet allows you to edit the peak arrival times in the Peak Finder Results table before clicking the [Accept] button. Contact Caliper’s Customer Service group if you need additional help (call 1-877-522-2447).

Peak Finder Results (Auto Mode)

Filename: \\Professor\Professor D drive\Hayley\AppData\2003-08-14\VISCMOB_CK2_2003-08-14_14-14-31.RDA

Accept Review the charts and edit the table (if needed), then click [Accept].

Sipper	Time of Occurrence of Peaks . . .									
1	135.3563	234.5563	334.0563	435.0563	578.6563	678.1563	767.0563	814.6563	914.0563	
2	138.7563	237.9563	337.4563	438.5563	584.6563	684.1563	766.0563	817.4563	916.8563	
3	134.7563	233.9563	333.3563	434.3563	576.1563	675.6563	767.2563	814.3563	913.7563	
4	135.1563	234.3563	333.8563	434.8563	577.6563	677.1563	767.2563	814.6563	913.9563	

Type the times for the correct peak in this column, then Click [Accept].

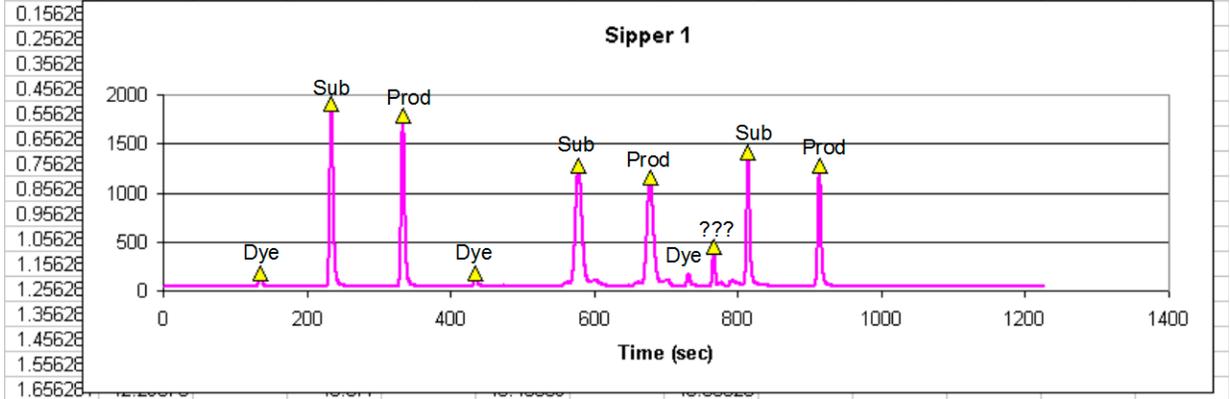


Figure 9. Example of a Viscosity/Mobility measurement file that cannot be analyzed automatically by the spreadsheet due to the extraneous peak (labeled “???”) near 800 sec. The user needs to enter the arrival time of the correct Dye peak into the table above the plot, and then click [Accept].

The most common source of “contamination” that leads to extra (interfering) peaks in the viscosity/mobility measurement data is contamination of the product by the substrate itself—in other words, incomplete product conversion (see Figure 10). If the contamination of the product by the substrate is small enough that the peak finder ignores the substrate peak, then the contamination will not interfere with the measurement and it can be safely ignored. However, if the product conversion is less than 50%, so that the contaminating substrate peak is taller than the product peak, corrective action must be taken. Perhaps the best thing to do is to make more product and repeat the measurement experiment. You may also edit the peak table to manually enter the arrival time of the correct (product) peak, as illustrated in Figure 9.

VISCMOB_CK2_2003-08-14_16-33-35.RDA

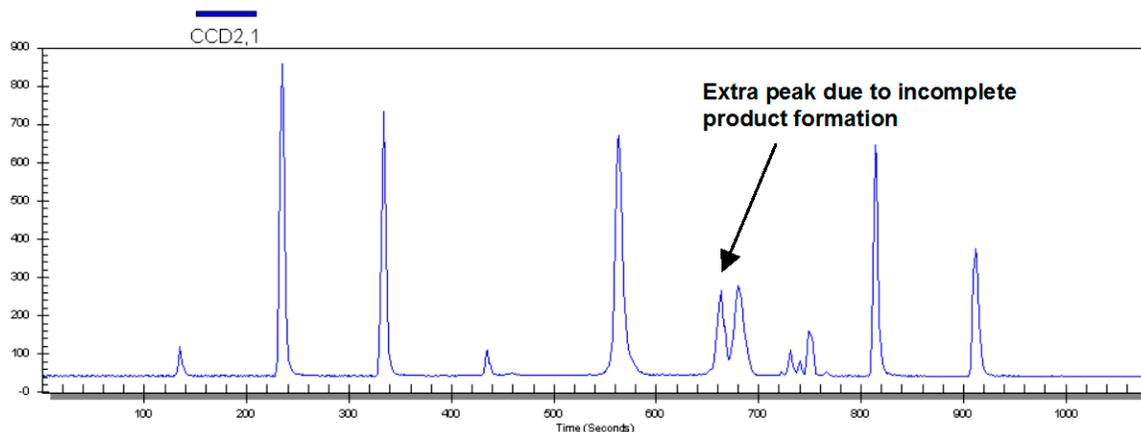


Figure 10. Example of double peak in the viscosity/mobility measurement data due to incomplete product conversion. If the contamination of the product by the substrate is small, it can be ignored. If the substrate part of the peak is greater than the product peak, corrective action needs to be taken.

3.2 Example using only 4-sippers of a 12-sipper chip.

The following example is provided to further illustrate the technique of editing the peak arrival table before clicking the [Accept] button, and also to provide a practical means of saving reagent costs during the assay development process.

As a means of saving reagent costs, it is possible to perform the viscosity/mobility measurement experiment using only four of the twelve sippers on an EZ Reader 12-sipper chip. To do this, follow the general experimental protocol detailed in Section 1.5, but augment those directions with the plate preparation and data analysis procedure described below.

Plate Preparation:

Prepare a 384 well microtiter plate as illustrated in Figure 11 for use with EZ Reader 12-sipper chips, where:

- N 70 μ l of neutral dye (1 μ M Bodipy Fluorescein Arginine, or 150 μ M Rhodamine B).
- Sub = 70 μ l of 1 μ M substrate peptide.
- Prod = 70 μ l of 1 μ M product peptide.
- S/P = Sub/Prod mixture: 70 μ L of 1 μ M product and 1 μ M substrate.
- B = 70 μ l of 1X separation buffer.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	N	N	N	N	B	B	Sub	Sub	Sub	Sub	B	B	Prod	Prod	Prod	Prod	B	B	S/P	S/P	S/P	S/P	B	B
B																								
C																								
D																								
E	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
F																								

Figure 11. Plate layout for using only 4-sippers of a 12-sipper chip to make viscosity/mobility measurements.

With the plate prepared as shown in Figure 11, only sippers 7, 8, 9, and 10 will participate in the experiment. The other sippers just sip buffer all the time. This presents a small problem for the data analysis macro since it will try to analyze the data from all 12 sippers. You can intervene to correct that by editing the peak arrival table. You just need to delete the peak times from all of the sippers that you want to ignore—leave blank entries in the table for sippers {1, 2, 3, 4, 5, 6, 11, and 12}. Then click the [Accept] button.

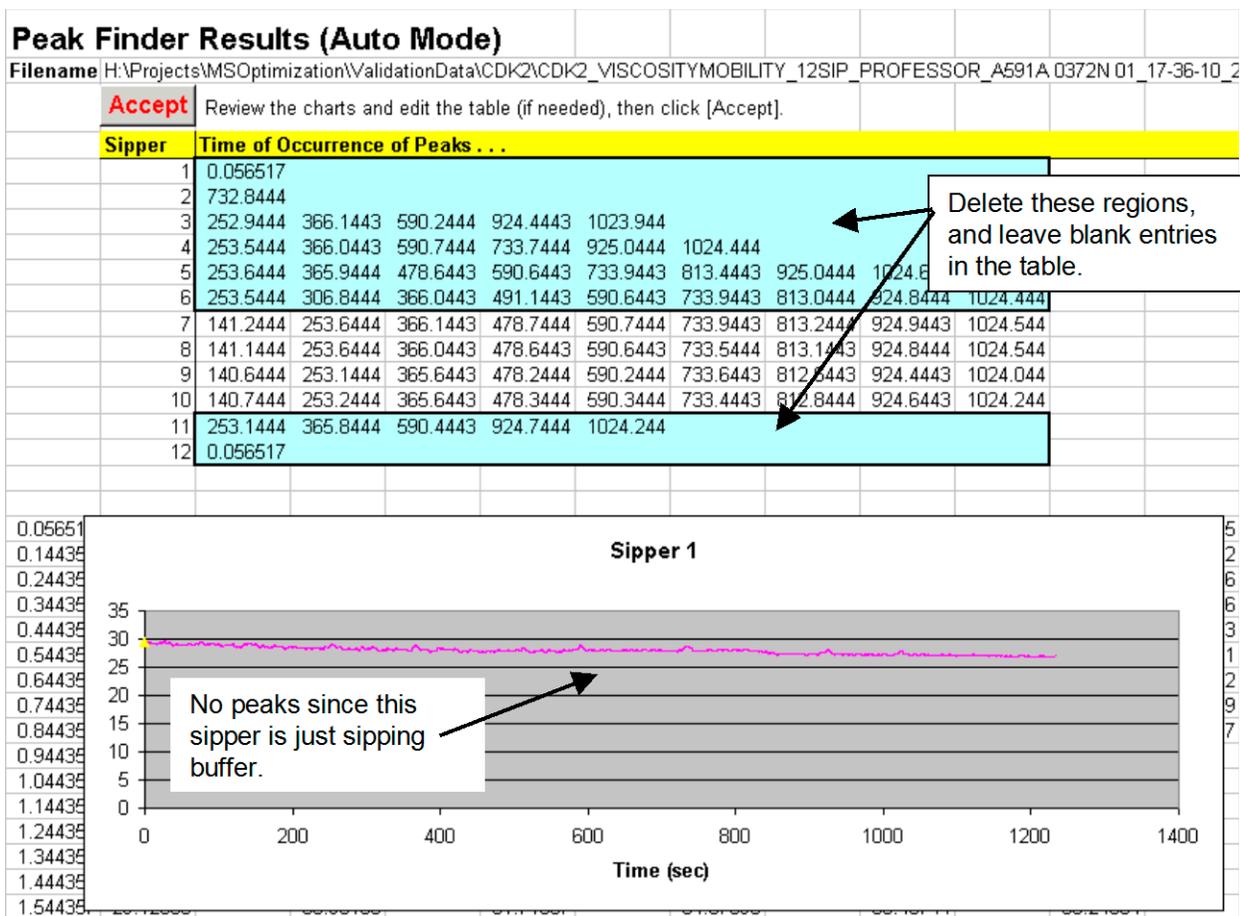


Figure 12. Illustration of a 12-sipper chip where only 4 sippers (7, 8, 9, and 10) are used to measure the viscosity and mobility. The user must edit the peak table by deleting the values for unused sippers, before clicking the [Accept] button.

3.3 Review the Viscosity/Mobility Measurement Results

Close inspection of the results obtained from the viscosity/mobility measurement experiment may reveal some common problems with the assay or buffer. A few things to look for are:

- Retention Factor:** Check to be sure the Substrate Retention Factor and Product Retention Factor are less than 1.03. The retention factor is the ratio of arrival time of the dye to arrival time of the substrate or product. It is a measure of the stickiness of the substrate/product relative to the dye. We assume the dye is not sticky, so a retention factor of 1 means the substrate or product is also not sticky. If the substrate or product solution tends to stick to the glass surface of the chip, the solution will move more slowly through the chip than the dye, so its retention factor will be greater than 1. A retention factor of something greater than 1.03 usually means the substrate or product is too sticky to use in a Mobility-Shift assay. Changing the buffer composition can solve most sticky problems. See the Off-Chip Mobility-Shift Assay Development Guide for more details on how to solve substrate/product sticking problems.
- Substrate and Product must have different mobility:** Sometimes the procedure used to convert the substrate into product fails so that no product is actually produced. In that case, the solution that you think is product is actually still just the substrate. If you perform the viscosity/mobility measurement with product that is really substrate, the spreadsheet will say that the product and substrate have about the same mobility.
- Electroosmotic Mobility:** The expected range of electroosmotic mobility (μEo) for a buffer containing about 0.1% Coating Reagent 3, is $\{-1\text{e-}5$ to $1\text{e-}5$ $\text{cm}^2/\text{V}\cdot\text{s}\}$. Measurements outside that range tend to indicate an error in the buffer composition or a failed experiment. Double check the buffer and repeat the measurement.

4. **Viscosity:** The range of buffer viscosity that is valid for use with the standard viscosity/mobility measurement job files is {1.2 cp to 1.8 cp}. A buffer viscosity measurement outside of that range may indicate an error in the buffer composition or a failed experiment. Double check the buffer composition and repeat the measurement. If you continue to observe viscosity measurements outside of the valid range, contact Caliper's Customer Support group for advice (call 1-877-522-2447). If you need to work with a buffer that has a viscosity outside of the range stated above, you will need to use a custom measurement job file since the timing of the voltage transitions depends on the buffer viscosity.

Run Analysis Macro		Auto Mode		Manual Mode																	
Experiment / Job Properties:																					
Filename	Chip	Applied Pressure (psi)	Measure Voltage1 (V)	Measure Voltage2 (V)	Initial Delay (s)	Buffer Dwell (s)	Sip Time (s)	Robot Movement Time (s)	Startup time (s)												
C:\Projects\EZ Reader		-1.5	-1400	1400	30	106	0.2	1.6	32.5												
Chip: EZ Reader 4-Sipper																					
Trial (channel)	Peak Arrival with:			...1st Voltage Applied			...2nd Voltage Applied														
	Neutral Peak Time (s)	Substrate Peak Time (s)	Product Peak Time (s)	Neutral Peak Time (s)	Substrate Peak Time (s)	Product Peak Time (s)	Neutral Peak Time (s)	Substrate Peak Time (s)	Product Peak Time (s)												
1	116.40	225.80	335.10	443.90	553.50	651.80	771.40	880.30	1013.90												
2	117.40	226.80	336.00	444.80	554.40	652.40	772.30	881.20	1015.80												
3	116.60	226.10	335.20	444.20	553.80	652.00	771.70	880.60	1014.70												
4	116.40	225.90	334.90	443.90	553.60	651.70	771.50	880.30	1014.20												
5																					
6																					
7																					
8																					
9																					
10																					
11																					
12																					
Mean:	116.70	226.15	335.30	444.20	553.82	651.98	771.73	880.60	1014.65												
										Electro-Mobilities (cm ² /V*s)											
										Sub μEp 1st V	Sub μEp 2nd V	Prod μEp 1st V	Prod μEp 2nd V	μEo 1st V	μEo 2nd V	Viscosity	Sub Retention Factor	R			
										Sipper-to-Detector Time (s):											
1	83.90	84.10	84.20	83.80	84.20	73.30	83.70	83.40	107.80	2.72E-06	2.09E-06	-1.02E-04	-1.00E-04	-6.87E-07	1.38E-06	1.38	1.00				
2	84.90	85.10	85.10	84.70	85.10	73.90	84.60	84.30	109.70	2.68E-06	2.06E-06	-1.03E-04	-1.01E-04	-1.35E-06	2.02E-06	1.39	1.00				
3	84.10	84.40	84.30	84.10	84.50	73.50	84.00	83.70	108.60	2.70E-06	2.07E-06	-1.02E-04	-1.01E-04	9.36E-11	6.83E-07	1.38	1.00				
4	83.90	84.20	84.00	83.80	84.30	73.20	83.80	83.40	108.10	3.40E-06	2.79E-06	-1.03E-04	-1.00E-04	-6.87E-07	6.87E-07	1.38	1.00				
5																					
6																					
7																					
8																					
9																					
10																					
11																					
12																					
Mean:	84.20	84.45	84.40	84.10	84.52	73.48	84.03	83.70	108.55	2.87E-06	2.25E-06	-1.02E-04	-1.01E-04	-6.80E-07	1.19E-06	1.38	1.00				
Results Output:																					
Estimated Viscosity (cP)	Substrate Mobility (cm ² /V*s)	Product Mobility (cm ² /V*s)	EO Mobility (cm ² /V*s)	Electrical Resistance (MΩ)	Substrate Retention Factor	Product Retention Factor	Mean Current (μA)	Observed Current1 (μA)	Observed Current2 (μA)	Observed Current3 (μA)	Observed Current4 (μA)	Observed Current5 (μA)	Observed Current6 (μA)								
1.38	2.56E-06	-1.02E-04	2.56E-07	201.03	1.00	1.00	6.96	6.94	6.99												
Summary Electro-Mobilities (cm ² /V*s)																					
		Sub μEp	Prod μEp	μEo																	
1st V		2.87E-06	-1.02E-04	-6.80E-07																	
2nd V		2.25E-06	-1.01E-04	1.19E-06																	
Mean		2.56E-06	-1.02E-04	2.56E-07																	
Difference		6.211E-07	1.628E-06	872E-06																	

Retention Factors must be less than 1.03.

Mobility must be different for substrate and product

Figure 13. Typical values measured in a Viscosity/Mobility experiment as shown on the *Experimental Estimates* worksheet. Close inspection of the results may reveal problems with the measurement or the buffer or reagent preparation.

Appendix A Mobility-Shift Assay Predictions Worksheet

A.1 Selecting the Resolution

In order to balance data quality and throughput, Caliper recommends:

- For an Off-Chip Incubation assay, set Resolution = 1.5, and Resolution Between Wells >= 2.0.

Where the Resolution refers to the substrate and product peaks, and the Resolution Between Wells refers to the resolution of the lagging peak of one well to the leading peak of the next well as illustrated in Figure 14.

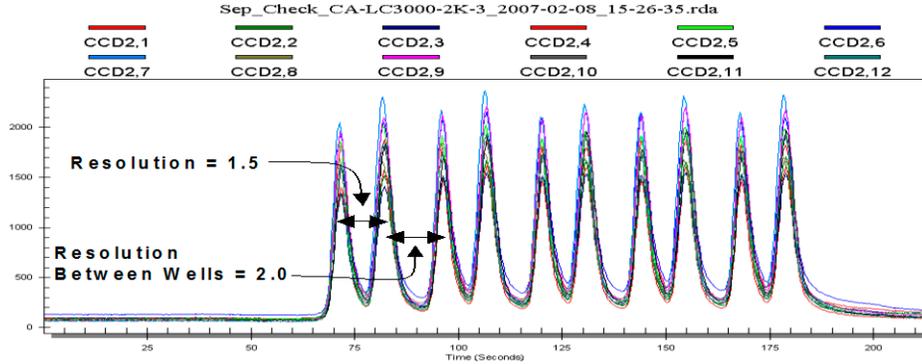


Figure 14. Example Mobility-Shift data illustrating the characteristics of Resolution = 1.5, and Resolution Between Wells = 2.0.

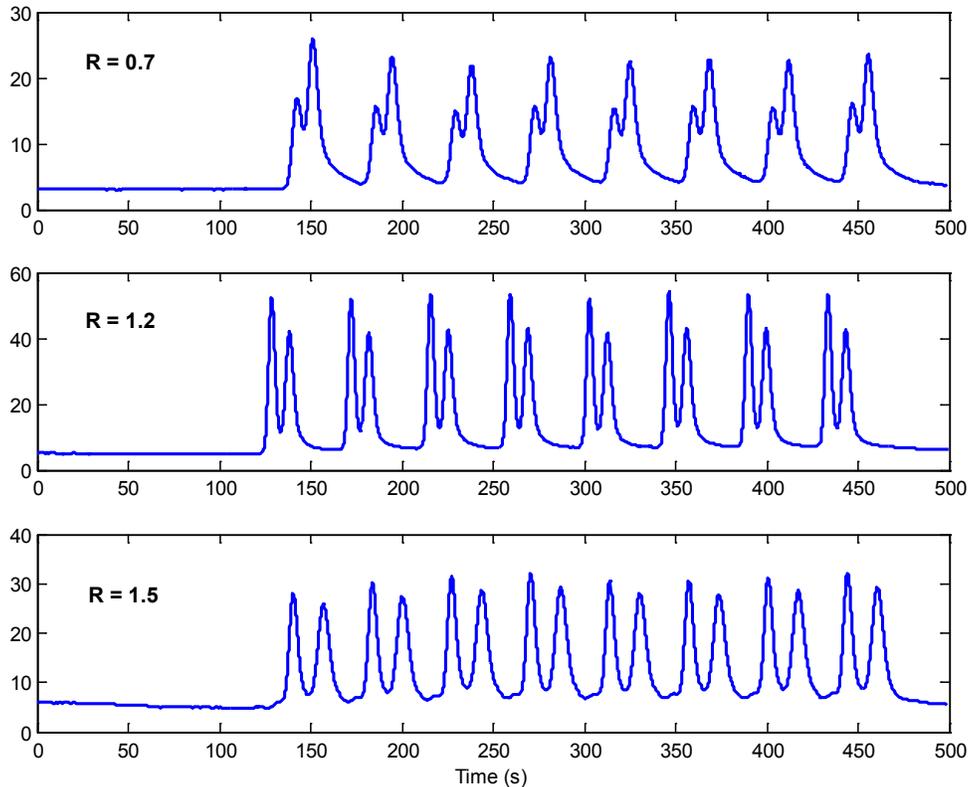


Figure 15. Several examples of Mobility Shift assay data to illustrate different resolutions of substrate and product peaks.

A.1.1 How is Resolution Defined?

$$R = \frac{\Delta T}{2(\sigma_1 + \sigma_2)}$$

Where ΔT is the distance in time between the two peaks, and σ is the standard deviation of the peak with the assumption that it is a Gaussian distribution. These terms are illustrated in Figure 16. It is often difficult to estimate σ_1 and σ_2 from experimental data, especially when the two peaks are partially overlapping such that the full width cannot be seen. An alternative definition that uses parameters that are more easily observed is:

$$R = \frac{2\Delta T}{1.7(FWHM_1 + FWHM_2)}$$

Where again ΔT is the distance in time between the two peaks, and FWHM is the Full Width at Half Maximum value for the peak, as illustrated in Figure 16.

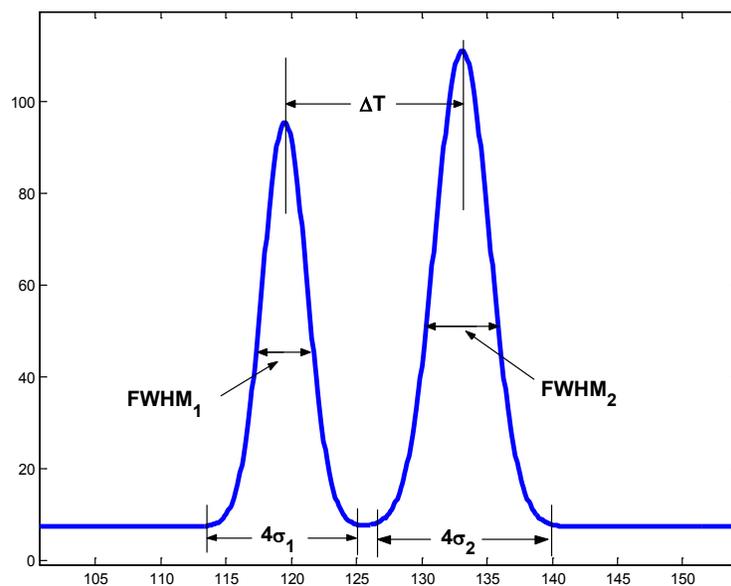


Figure 16. Illustration of terms for the definition of resolution used in Mobility-Shift Assays.

A.1.2 Practical Hints on Selecting the Resolution

When selecting the resolution for your particular assay, there are several issues to consider: i) the minimum resolution required by the data analysis software is $R = 1.2$; ii) there will be some variability in the resolution over time; iii) the resolution will impact the throughput; and iv) given the limits of performance of the instrument it may not always be possible to achieve the resolution that you desire. Let's consider some of these topics in more detail.

- **Plan for Variability in the Resolution:** If the desired resolution is set to some value R and the recommended voltage and pressure are used, it is likely that the resolution actually achieved when the assay is performed will have some error (or variation) about R . The main sources of error are: i) Variation in temperature (which will change the buffer viscosity); ii) Preparation of the reagents, iii) Change in pH of the buffer over time; iv) Normal manufacturing tolerances of the chips, and v) Measurement error when measuring the viscosity and mobility of the reagents. The assay developer should anticipate $\pm 10\%$ to $\pm 20\%$ variation in the resolution, depending on the degree of control that is placed on the sources of error. To make a robust assay that is insensitive to changes in the variables listed above, it is best to factor in some margin of safety when selecting the resolution. The assay developers at Caliper follow the convention of setting the *Desired Resolution* = 1.5 and the *Resolution Between Wells* ≥ 2.0 . These values have been chosen since the lower limit on the resolution that Caliper's data analysis software can reliably process is $R=1.2$, and we want to allow for a $\pm 20\%$ worst case variation in the resolution from day-to-day (due to the combination of all error sources). By setting the desired Resolution = 1.5, and the Resolution Between Wells ≥ 2.0 , we allow the resolution to vary a little bit from day-to-day without affecting the results obtained from the data analysis software.
- **Choice of Resolution will Affect Throughput:** Since the substrate and product are spatially separated inside the chip, we are required to sip buffer for some period of time between each sample, to ensure that adjacent samples do not interfere with each other. If the resolution of the substrate and product is small, their peaks will be close together so the required inter-sample buffer sipping time can be small. However, if the resolution is large, the substrate and product will be farther apart so the required buffer sipping time will be larger. In short, the price that must be paid for higher resolution is lower throughput, so it usually does not make sense to run an assay at a resolution greater than 1.5 unless you expect more than about $\pm 20\%$ variation from run-to-run.

A.2 Troubleshooting Resolution Errors

Figure 17 shows several examples of Separation Check files produced by different assays (i.e., different peptides). Although the plots look somewhat different from each other, they are all considered acceptable, and the assays have all been used in successful screens. The differences in the plots are mainly due to the different mass and charge of the peptides, which causes more or less dispersion of the peaks, and, to a lesser extent due to small differences in the resolution of the peaks. If you produce a Separation Check file that is unacceptably different than the examples shown here, follow the steps listed below, to try to correct the problem.

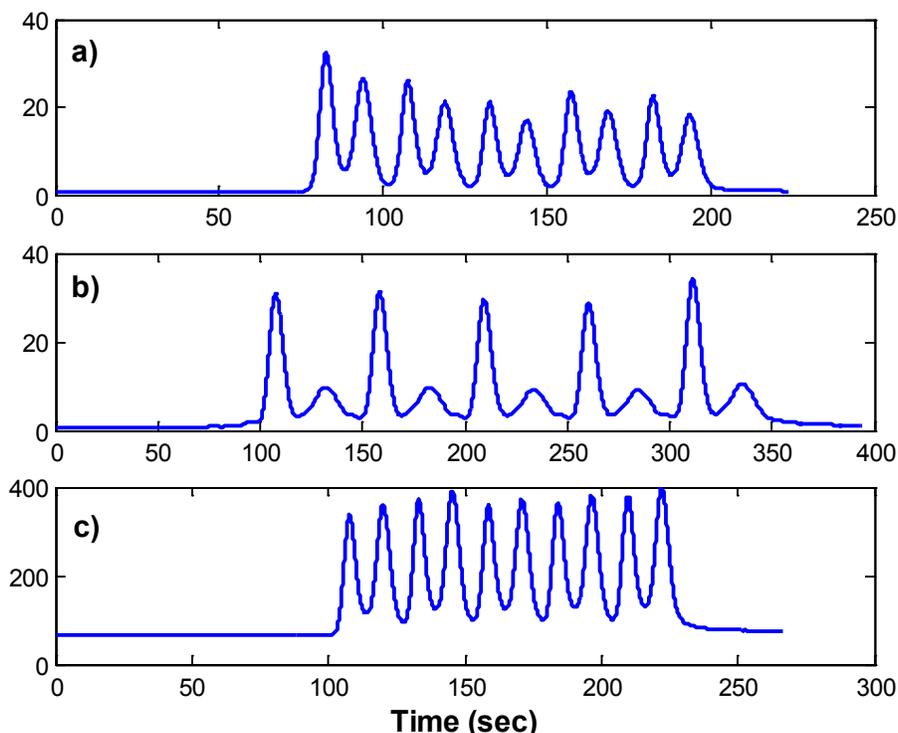


Figure 17. Examples of acceptable Separation Check files for several different assays: a = Fyn, b = CK2, c = PRAK. The assays each have different shaped peaks due to differences in the mass and charge of the peptides used. Even though they look somewhat different, they are all considered acceptable, and the assays have given good performance.

What if I do not actually achieve the resolution that the spreadsheet predicts?

The most likely reason for errors in the predicted resolution is a poor estimate of the viscosity or mobility. Try some of the following things to improve the measurements:

1. Double check the *Peak Finder Results* worksheet looking for anomalies like extra peaks in the data signal that might have interfered with the analysis of the data (see Section 2.1 and Figure 9).
2. Review the viscosity/mobility measurement results on the *Experimental Estimates* worksheet as described in Section 2.3 and Figure 13. Look for things like incomplete product formation, sticky substrate/product, or viscosity outside of the expected range.
3. Be sure that the buffer you used for the viscosity/mobility measurement experiment is actually the same buffer that you are running the assay in. If you change any of the ingredients in the buffer, you must repeat the viscosity/mobility measurement experiment using the new buffer.
4. Control the Temperature: Viscosity and mobility are temperature dependent. Be sure that you perform the viscosity/mobility experiment at the same temperature as you run the assay. Be sure to allow the chip and buffer to reach the ambient temperature before performing the viscosity/mobility measurement.
5. Double check the Chip Type: Be sure that you perform the viscosity/mobility experiment using the same type of chip as you will use to run your assay. For example, if you want to run your assay using an EZ Reader 12-sipper chip, you must use an EZ Reader 12-sipper chip to perform the viscosity/mobility measurement experiment.
6. Just repeat the viscosity/mobility measurement experiment.
7. If after considering the things listed above, you still observe poor separation of peaks in the Separation Check file, contact Caliper's Customer Service Group (by calling 1-877-522-2447) for advice. Be prepared to email us a copy of the spreadsheet you are using and the data file produced by your viscosity/mobility measurement experiment.

A.3 Interpreting the Input Data Table

Definitions of the input data table on the *Experimental Assay Predictions* worksheet:

Chip – Use the drop down menu to select the type of chip that you will use for your assay. For any particular assay, the best operating conditions of voltage, pressure, and Buffer Dwell will, in general, be different for each chip type.

Viscosity – The viscosity of the assay buffer in centi-poise (cp). The user may type in any value for a hypothetical simulation; however, to obtain useful predictions of operating parameters for a particular assay, the viscosity must be measured using the viscosity/mobility experiment described in Section 1.5

Substrate/Product Electrophoretic Mobility – When placed within an electric field, an electrically charged solute will migrate through a buffer with an electrophoretic velocity $U_{EP} = \mu_{EP}E$ (cm/s). Where μ_{EP} is the electrophoretic mobility and E is the applied electric field. Separation is achieved because the substrate and product migrate through the chip at different velocities. The user may type in any value for the substrate and product electrophoretic mobility to perform a hypothetical simulation, however, for best results when developing an assay for a particular substrate and product peptide, the electrophoretic mobility must be measured using the viscosity/mobility estimate experiment described in Section 1.5.

Electroosmotic mobility – When an electric field is applied across the LabChip's separation channel, the buffer will be set in motion with a velocity $U_{Eo} = \mu_{Eo}E$ (cm/s). Where μ_{Eo} is the electroosmotic mobility and E is the applied electric field. The user may type in any value for a hypothetical simulation, however, to obtain useful predictions of operating parameters for a particular assay, the electroosmotic mobility must be measured using the viscosity/mobility experiment described in Section 1.5.

Substrate/Product Molecular Diffusivity – The molecular diffusivity coefficient is used by the spreadsheet to predict the amount of spreading (or widening) the sipped samples will experience as they pass through the chip. Short peptides typically have a molecular diffusivity value of about 1×10^{-6} cm²/s. The user may type in any value for the substrate and product molecular diffusivity, however, for best results when developing an assay for a particular substrate and product peptide use the "A Priori Estimates" worksheet to estimate the values based on the amino acid sequence as described in Section 1.3.

pH – pH of the buffer. This information is needed for the buffer lifetime calculation.

[Buffer1,2,3] – Concentration of buffering species (needed for the buffer lifetime calculation). The user may provide values for up to three buffering components in the buffer.

pK1,2,3 – pK values for up to three buffering components (needed for the buffer lifetime calculation).

Resistance of Separation Circuit – The electrical resistance of the chip's separation circuit. The resistance is a function of the chip geometry as well as the conductivity of the buffer. This value is measured as part of the viscosity/mobility measurement experiment.

Robot Movement Time – This is the "Travel Time" stated in the RDA file divided by 2 (since the RDA file states the "round trip robot travel time"). The Robot Movement Time is measured as part of the viscosity/mobility measurement experiment. It is typically about 1.5 seconds.

Sample Sip Time – The "Sample Dwell" time as set in the instrument Experiment/Job file (typically 0.2 sec. for a Mobility-Shift assay).

A.4 Interpreting the Output Data Table

Definitions of the output data table on the *Experimental Assay Predictions* worksheet:

Voltage - The voltage values listed in the output table are the difference of potential to be applied between the upstream electrode well and the downstream electrode well. Where the positive voltage is defined as the one that will propel a positive charge from the entrance of the separation channel toward the detector. Therefore, with a positive voltage applied, the upstream electrode will be positive with respect to the downstream electrode.

If the desired resolution cannot be achieved by applying a voltage within its search range, the function that solves for the voltage returns "Impossible", meaning it is impossible to achieve to desired resolution. The function that solves for the voltage searches within the range of 200V to POSITIVE_VMAX and - 200V to NEGATIVE_VMAX. Where, POSITIVE_VMAX is equal to the highest voltage that can be applied without producing a total loss of the charged species to the Upstream Electrode, or ABSOLUTE_VMAX whichever is smaller. NEGATIVE_VMAX is equal to the largest negative voltage that can be applied without producing a total loss of the charged species to the Upstream Electrode, or - ABSOLUTE_VMAX whichever has the smaller magnitude. Where ABSOLUTE_VMAX is the maximum value that can be produced by the power supply. By limiting the search range in this way, the spreadsheet avoids recommending a voltage that is outside the limits of performance of the instrument or that would produce a loss of the substrate or product to the upstream electrode well.

Post Sample Buffer Sip Time - The length of time required for the instrument to sip buffer between each sample to produce desired "Resolution Between Wells". The Resolution Between Wells is defined as the resolution of the lagging peak of one well to the leading peak of the next well. You need to consider the Resolution Between Wells to ensure that adjacent samples will not interfere with each other. The Post Sample Buffer Sip Time is a parameter that must be set in the instrument Experiment/Job file.

Post Dye Buffer Sip Time – The length of time required for the instrument to sip buffer after each row marker (dye) so that the row marker does not interfere with the first sample of the row. This is another parameter that must be set in the instrument Experiment/Job file.

Final Delay – The length of time required for the instrument to sip buffer at the end of a plate, so that the last sample sipped (usually a row marker) has enough time to flow through the chip, past the detector and allow the signal to return to baseline before the instrument stops recording data. The Final Delay is a parameter that must be set in the instrument Experiment/Job File.

Time for One Plate – Approximate time required to run one 384 well plate. This calculation accounts for the Sample Sipping Time; Post Sample Buffer Sipping Time, Robot Movement Time, Row Marker Sipping Time, and the Final Delay; and it adjusts the time appropriately depending on the type of chip (4-sipper, 12-sipper, etc).

Peak Order – The order in which the substrate and product peaks will appear in the output data file from the Caliper HTS instrument: Product First or Substrate First. This information is provided to make it more convenient for the user to set the Peak Order in the data analysis software.

Buffer Lifetime - The Buffer Lifetime is the amount of time it will take for the buffer in the electrode well to change by 1 pH, due to the electric current passing through it (see Figure 18). The function implemented in the spreadsheet estimates the time for the upstream electrode and the downstream electrode then returns whichever value is smaller. The calculation assumes that the electrode wells on the chip have been filled with the recommended volume of buffer. Be sure to fill the wells correctly to achieve the predicted buffer lifetime.

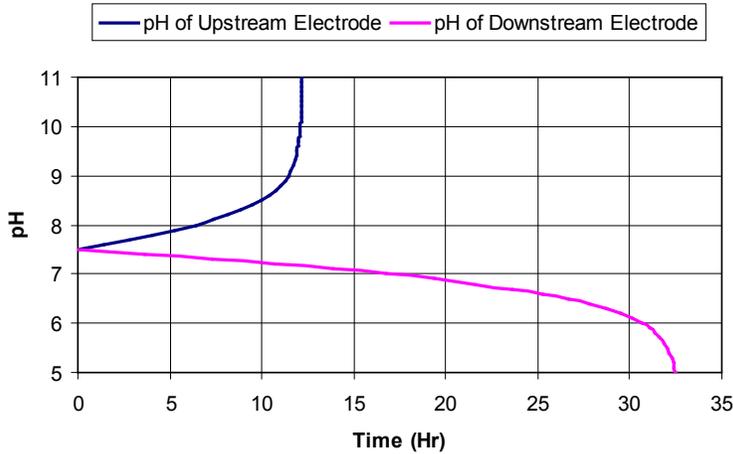


Figure 18. Illustration of the change in pH of the Upstream and Downstream electrode wells over time for an EZ Reader 12-sipper chip with 100mM HEPES buffer. The Buffer Lifetime is defined as the amount of time it will take for the pH to change by 1pH unit (about 10 hours in this example).

Appendix B Mobility-Shift Calculator Worksheet

The *Mobility-Shift Calculator* worksheet is provided as an aid to perform more detailed analysis of an assay if you wish. The calculator allows you to input any arbitrary set of input parameters (like viscosity, mobility, voltage, pressure, etc) and it will predict the expected peak separation, resolution, concentration factors, etc. Our intent is to allow the user to customize this worksheet (it's just an Excel Spreadsheet after all) to suit their interests and needs for various different analyses. For example, you might use this sheet to perform a sensitivity analysis on the viscosity—to see how much the resolution would change for your assay due to the change in viscosity that accompanies changing temperature in your lab.

Mobility-Shift Calculator								Caliper LifeSciences								
Use this worksheet if you wish to do more detailed analysis of various operating conditions. You can use this sheet to predict many interesting performance characteristics given any arbitrary set of input conditions. Try changing any of the inputs (voltage, pressure, viscosity, mobility, etc.), and see how the changes affect the performance of your assay.																
Version = 3.3.28.0; 3.2.0; OffChip																
Chip	Viscosity (cP)	Sub Mobility (cm/V*s)	Prod Mobility (cm/V*s)	EO Mobility (cm/V*s)	Sub Diffusivity (cm ² /s)	Prod Diffusivity (cm ² /s)	Sip Time (s)									
EZ Reader 12-	1.38	2.56E-06	-1.02E-04	2.56E-07	1.17E-06	1.15E-06	1.75									
Model Predictions:																
Pressure (psi)	Voltage (V)	dT (sec)	sigma_s	sigma_p	Resolution	Separation Time (s)	Sip-to-Detect Time (s)	Conc_S Factor	Conc_P Factor	Disp_S Factor	Disp_P Factor	Total_S Intensity	Total_P Intensity	FWHM_S (sec)	FWHM_P (sec)	Peak Order
-1.7	-300	2.37	1.78	1.71	0.34	32.44	74.37	0.63	0.58	0.39	0.41	0.246	0.237	4.18	4.02	Prod First
-1.7	-400	3.10	1.78	1.69	0.45	32.46	74.40	0.63	0.57	0.39	0.41	0.246	0.235	4.18	3.97	Prod First
-1.7	-500	3.78	1.78	1.67	0.55	32.48	74.42	0.63	0.55	0.39	0.42	0.246	0.232	4.18	3.92	Prod First
-1.7	-600	4.44	1.78	1.65	0.65	32.51	74.44	0.63	0.54	0.39	0.42	0.246	0.230	4.18	3.87	Prod First
-1.7	-700	5.08	1.78	1.63	0.74	32.53	74.47	0.63	0.53	0.39	0.43	0.246	0.227	4.19	3.83	Prod First
-1.7	-800	5.68	1.78	1.61	0.84	32.55	74.49	0.63	0.52	0.39	0.43	0.246	0.225	4.19	3.78	Prod First
-1.7	-1000	6.82	1.78	1.58	1.01	32.60	74.54	0.63	0.50	0.39	0.44	0.246	0.220	4.19	3.71	Prod First
-1.7	-1400	8.86	1.79	1.53	1.33	32.69	74.63	0.63	0.46	0.39	0.46	0.247	0.210	4.20	3.60	Prod First
-1.7	900	-9.88	1.77	2.22	1.24	32.16	74.10	0.62	0.81	0.39	0.31	0.245	0.255	4.15	5.23	Sub First
-1.7	1200	-14.60	1.76	2.48	1.72	32.09	74.03	0.62	0.90	0.40	0.28	0.245	0.254	4.15	5.82	Sub First
-1.7	1500	-20.48	1.76	2.82	2.23	32.03	73.96	0.62	1.00	0.40	0.25	0.245	0.247	4.14	6.63	Sub First
-1.7	1900	-31.01	1.76	3.53	2.93	31.94	73.87	0.62	1.00	0.40	0.20	0.245	0.198	4.13	8.29	Sub First
-1.7	2600	-64.75	1.75	6.35	4.00	31.78	73.72	0.61	1.00	0.40	0.11	0.244	0.110	4.12	14.92	Sub First

Figure 19. Example of the Mobility-Shift Calculator worksheet.

B.1 Definition of Output Values

dT - The time difference between the substrate and product peaks.

Resolution - The resolution of the substrate and product peaks, as defined in Section A.1.1.

Incubation Time - The Incubation Time is defined as the length of time it will take for a fluid element to pass through the chip's incubation channel (from input to output). For an On-Chip incubation assay, the Incubation Time is the length of time that the enzyme, substrate, and sample are allowed to react before arriving at the entrance to the separation channel.

Note that the EZ Reader 4- and 12-sipper (for the Off-Chip Incubation assay) do not have an incubation channel, so the incubation time is reported as zero seconds.

Separation Time - The Separation Time is defined as the average length of time it will take for a Substrate molecule to travel the length of the separation channel, at the given voltage and pressure. This calculation includes the effects of the pressure driven flow, electroosmotic flow, and electrophoretic mobility of the substrate molecules.

Sip-toDetect Time - The average length of time it will take for a Substrate molecule to travel from the tip of the sipper to the detector, at the given voltage and pressure.

Substrate (Product) Concentration Factor - If the concentration of substrate is 1 just before entering the separation channel, the concentration will be Conc_S (Conc_P) at the detector.

A change of concentration may occur due to dilution by Electroosmotic Flow entering from the Upstream Electrode channel and/or due to a change in velocity of the species as it enters the separation channel (recall that velocity and concentration are inversely related by the principle of conservation of mass flux).

Substrate (Product) Dispersion Factor: Disp_S (Disp_P) - Predicts the normalized peak magnitude of a short sipped slug which has been spread by dispersion due to the pressure driven flow, as the slug has travels through the chip from sipper-to-detector. If there were no dispersion, the dispersion factor would be 1.

Full Width at Half Maximum - The width of the substrate (product) peak at half of its maximum height.

Peak Order - The order in which the substrate and product peaks will appear in the output data file when the assay is run: Product First or Substrate First. This will help identify which peak is the product and which peak is the substrate as observed in the data file.