Alpha *SureFire*® CETSA® Kit

Assay Kits Generic Manual

Assay Points	Catalog #
500	CETSA-XXXX-X500
5 000	CETSA -XXXX-X5KX

This Manual is a generic manual for all the Alpha *SureFire*® CETSA® kits. For target-specific information, relating to Kit Specificity, Control Lysates and Representative Data, please refer to the Technical Data Sheet of the kit, also available from www.perkinelmer.com

For research use only. Not for use in diagnostic procedures.

For an electronic version of this manual, please go to:
http://www.perkinelmer.com/category/alpha-CETSA-kits

Alpha *SureFire*® CETSA® kit

Assay Principle

The Cellular Thermal Shift Assay (CETSA) assesses the thermal stability of proteins in living cells and cell lysate based on denaturation and aggregation upon heating. The relative amount of remaining soluble protein after heating can be measured, and a thermal melting curve of a known target protein can be generated. Compound binding often affects the thermal stability of proteins, and the shift in the melting curve is indicative of cellular target engagement. Target engagement by compound binding can result in thermal stabilization of the protein target, leading to a right-shifted thermal denaturation profile (as exemplified in Figure 1), but can also result in protein destabilization, then leading to a left-shifted thermal denaturation profile.

The CETSA® assay is run by incubating intact cells or disrupted cells with the test compound and the assay will reflect the ability of the compound to interact with the target protein in a cellular context. In the case of intact cells, the CETSA® assay data is affected by cellular metabolism and permeability. The CETSA® assay takes into account the complexity of the cellular context, and as such provides very valuable and physiologically relevant target engagement information.

Typically, a melting curve is first generated, where the sample, in the presence and in the absence of a reference compound, is heat challenged at 12 different temperatures. From the melting curve a single temperature is selected, where about 80-90% of the target signal is lost, and where there is an obvious shift of the reference compound. This single temperature is then used to perform single concentration or concentration-response curve compound screening.

The concentration-response experiment determines the potency as the concentration yielding 50% of the maximal stabilization or destabilization effect (EC_{50}) at a single selected temperature. This EC_{50} value is in the literature also sometimes referred as “isothermal dose–response fingerprint” ($ITDRF_{CETSA}$), to signify its known dependence on assay conditions. The CETSA® assay EC_{50} can be used for ranking of compounds and reflects the direct compound binding to the native protein target in a cellular context. This data can be used for SAR analysis and correlation with other assay data.

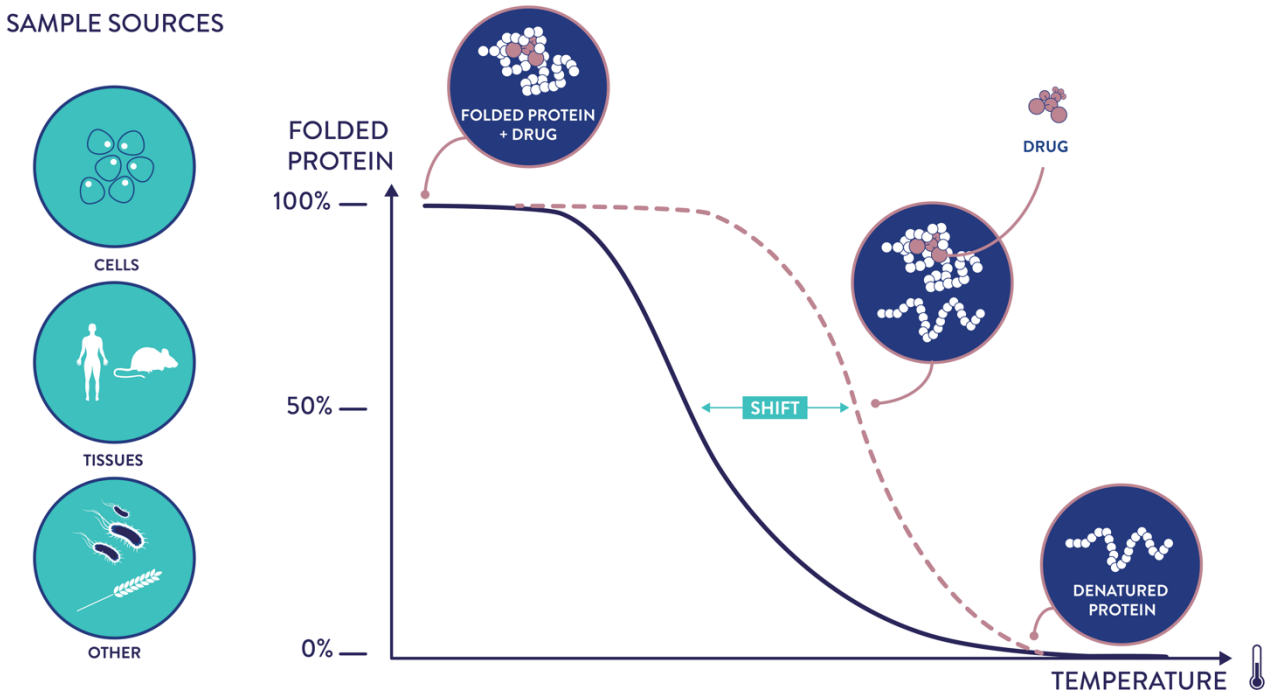


Figure 1 : Principle of the CETSA® melting curve, and thermal stabilization by compound binding.

The Alpha *SureFire*® CETSA® assay kits allow the rapid, sensitive and quantitative detection of the target protein remaining soluble after heat treatment of compound-treated cells. The kits utilize Alpha Beads that are each coated to specifically capture one of the assay antibodies (Figure 2). The Donor Bead is coated with streptavidin to capture the biotinylated antibody. The Acceptor Bead is coated with a proprietary Capt*Sure*™ agent that immobilizes the other assay antibody which is labeled with a Capt*Sure*™ tag.

In the presence of the target protein, the two antibodies bring the Donor and Acceptor Beads close to each other, enabling the generation of an Alpha signal upon illumination of Donor Beads by the Alpha-enabled plate reader, such as the EnVision® Multilabel Plate Reader, the EnSight™ or the Victor Nivo® Multimode Plate Readers. The amount of light emission is directly proportional to the amount of soluble protein present in the sample.

This assay eliminates the need for laborious techniques, such as Western blotting or conventional ELISA. It is a homogeneous assay, in that no sample washing steps are required, which allows for minimal handling, short assay times, better well-to-well reproducibility (lower CV%), and robotic operation if desired. The assay utilizes the bead-based Alpha Technology, and requires an Alpha Technology-compatible plate reader.

License

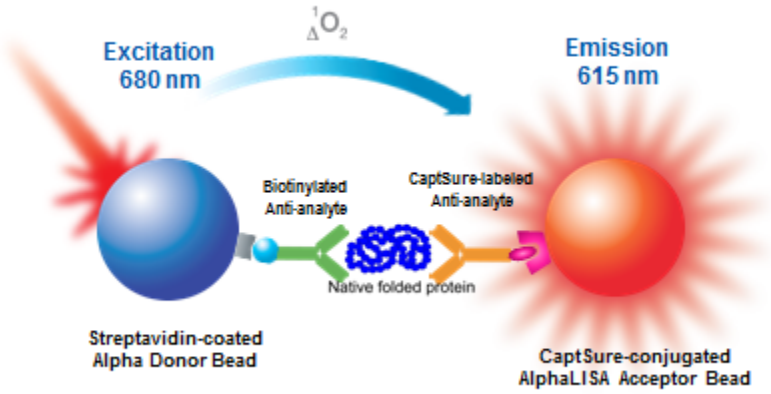
Please note that CETSA® is a registered trademark of Pelago Bioscience AB who hold the exclusive rights to the CETSA® patent family. If you do not have yet a valid license, please contact Pelago Biosciences at sales@pelagobio.com to discuss your needs.

The CETSA® method is patented in the following territories

United Kingdom:	Reg.no. 2490404
US:	Reg.no. 8969014, 9523693 and 9528996
Singapore:	Reg.no. 194137
China:	Reg.no. ZL201280025677.X
Korea:	10-1940342
Hong Kong:	HK1192612
India:	311112
Japan:	6032715
Australia:	2012246069
European states:	Austria, Belgium, Switzerland, Germany, Denmark, Spain, Finland, France, Great Britain, Ireland, Italy, the Netherlands and Sweden with Reg.no. 2699910

The granted and pending patents are based on patent application PCT/GB2012/050853.

A.



B.

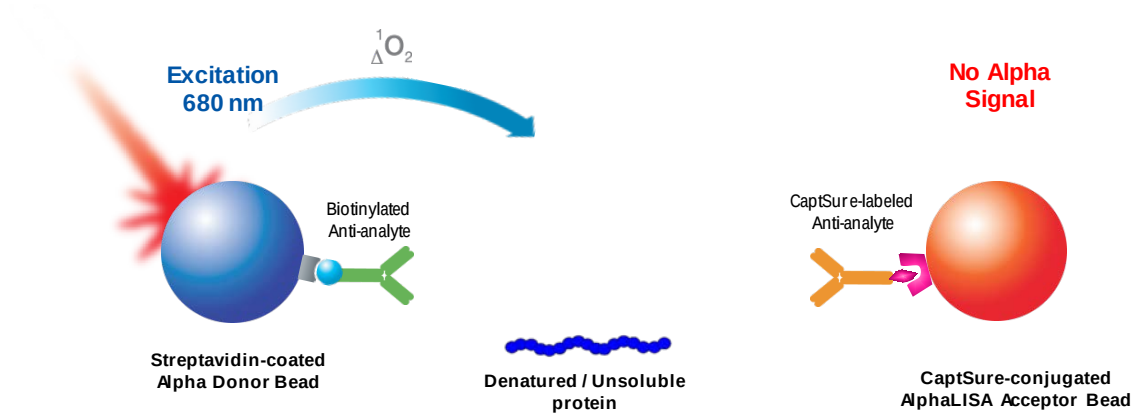


Figure 2 : Principle of detection of the soluble protein by the Alpha *SureFire*® CETSA® assay. Only the soluble protein will lead to the generation of an Alpha signal (A), while the thermally denaturated unsoluble protein is unable to create the proximity needed for the generation of an Alpha signal (B).

Kit-Specificity Information / Control Lysate Information / Representative Data

See Technical Data Sheet included in assay kit box.
Technical Data Sheets are also available as pdf file from
http://www.perkinelmer.com/category/alpha-CETSA-kits.

Please note that the Control lysates are provided as a control of the AlphaLISA® immunoassay (i.e., to control the ability of the reagents to detect the target), but that the Control lysate should not be used to perform melting curves, or compound stabilization experiments, as they do not present the required characteristics for being heat-challenged successfully.

Kit Contents

	Kit Size	
	500 points	**5,000 points**
CETSA Cell Lysis Buffer 1 (5X)	1 x 10 mL	2 x 50 mL (-K1 kit)
CETSA Cell Lysis Buffer 2 (5X)	1 x 10 mL	OR 2 x 50 mL (-K2 kit)
CETSA Cell Lysis Buffer 3 (5X)	1 x 10 mL	OR 2 x 50 mL (-K3 kit)
CETSA Cell Lysis Buffer 4 (5X)	1 x 10 mL	OR 2 x 50 mL (-K4 kit)
CETSA Cell Lysis Buffer 5 (5X)	1 x 10 mL	OR 2 x 50 mL (-K5 kit)
Activation Buffer - *Ultra*	1 x 0.8 mL	1 x 5 mL
Reaction Buffer 1 - *Ultra*	1 x 1.5 mL	1 x 14 mL
Reaction Buffer 2 - *Ultra*	1 x 1.5 mL	1 x 14 mL
Dilution Buffer - *Ultra*	1 x 3 mL	1 x 30 mL
AlphaLISA® Capt*Sure*™ Acceptor Beads (2mg/mL in PBS plus 0.05% Proclin-300)	1 x 0.06 mL	1 x 0.55 mL
Alpha Streptavidin Donor Beads (2mg/mL in PBS plus 0.05% Proclin-300)	1 x 0.06 mL	1 x 0.55 mL
Positive Control Lysate *	1 X Lyophilized tube (to be re-constituted with 250 µL H_2O)	

* Not to be used in Thermoshift assay, only for controlling the immunoassay performance

The above volumes supplied are in excess to the actual volume required to perform assay.

The choice of the **Cell Lysis Buffer** is an important part of the optimization of the CETSA® assays, and therefore 5 different Cell Lysis Buffers are provided in the 500 datapoints kit to experimentally determine which buffer is optimal for a particular cellular context. Once the optimal buffer has been found, 5 different versions of the 5000 datapoint kits are available, each containing a different Cell Lysis Buffer. The final number in the part number of these kits indicates which Cell Lysis Buffer is included in each of these kits. For example the CETSA-XXXX-X5K1 part number indicates that the kit contains CETSA Cell Lysis Buffer #1, and the CETSA-XXXX-X5K3 part number indicates that the kit contains CETSA Cell Lysis Buffer #3. Extra CETSA Cell Lysis buffers can be ordered separately (cat # CETSA-BUF1-100ML, CETSA-BUF2-100ML, CETSA-BUF3-100ML, CETSA-BUF4-100ML, CETSA-BUF5-100ML).

The CETSA Cell Lysis Buffers 1 to 5 all contain a proprietary mixture of pH buffers, detergents, and salts. The different CETSA Cell Lysis Buffers each use different types and concentrations of detergents, contain various concentrations of glycerol, or no glycerol, contain divalent cation chelators or not, contain different types of pH buffers and range from pH 7.0 to 7.5, and contain different types and concentrations of salts, in order to provide a variety of Cell Lysis Buffers so that an optimal one can be found for each target or cell type. The optimal Cell Lysis Buffer for a particular type of sample and target will need to be tested on a case-by-case basis.

The CETSA Cell Lysis Buffers do not contain protease inhibitors as they are commonly not needed to perform CETSA® assays; however, when working with sample types particularly rich in proteases (such as pancreatic cells) the addition of protease inhibitors to the CETSA Cell Lysis Buffers may be considered.

The table below highlights key differences between the Lysis Buffers.

Cell Lysis Buffer #	**Key Considerations**
CETSA Cell Lysis Buffer 1	Most aggressive detergent formulation, contains divalent cation chelators, low salt concentration, moderate glycerol concentration
CETSA Cell Lysis Buffer 2	Less aggressive detergent formulation; optimized for lysis of a broad range of cells without releasing nuclear DNA and minimally disrupting protein interactions; physiological salt concentrations
CETSA Cell Lysis Buffer 3	Medium detergent concentration, strongest pH buffering capacity, no glycerol, low salt, 0.1% casein
CETSA Cell Lysis Buffer 4	Medium-High detergent concentration, contains divalent cation chelators, close to physiological osmotic strength, no glycerol
CETSA Cell Lysis Buffer 5 *	Medium-High detergent concentration, contains divalent cation chelator, close to physiological osmotic strength, highest glycerol concentration Please note that the CETSA Cell Lysis Buffer 5 may turn yellow over time, which has no impact on assay performance.

All Alpha *SureFire*® CETSA® kits contain the same formulations of **Cell Lysis Buffers (5X)**, **Dilution Buffer - *Ultra***, **Activation Buffer - *Ultra*, Acceptor** and **Donor Beads** and <u>are</u> interchangeable between kits. The **Reaction Buffers** contain assay-specific antibodies and the **Control Lysates** are assay specific and <u>are not</u> interchangeable.

* Please note that the CETSA Cell Lysis Buffer 5 may turn yellow over time, which has no impact on assay performance.

Storage Conditions (See kit box label for expiry date)

Unopened kit	Store at 4°C. DO NOT freeze the kit. The Reaction Buffers contain antibodies and freeze/thaw cycles can lead to a loss of activity.	
Opened	CETSA Cell Lysis Buffers 1 to 5 (5X)	Store at 4°C
	Reaction Buffer 1 - *Ultra*	
	Reaction Buffer 2 - *Ultra*	
	Dilution Buffer - *Ultra*	
	Acceptor Beads	
	Activation Buffer - *Ultra*	Precipitates at 4°C. To re-dissolve, warm to 37°C and mix before each use. Alternatively, can be stored at room temperature with no loss in activity.
	Donor Beads	Store at 4°C in the dark, and should be returned to the kit box after use.
	Positive Control Lysate	Store at 4°C or for long term storage at -80°C

Materials Required But Not Provided

Item	Suggested source	Catalog #	Size
HardShell PCR Plate, 96 wells, Blue	PerkinElmer Inc.	6008870	50/box
HardShell PCR Plate, 384 wells, Blue	PerkinElmer Inc.	6008910	50/box
TOPSEAL-B FOR PCR PLATE	PerkinElmer Inc.	6050174	100/box
HBSS (1x) Hank's Balanced Salt Solution (with $CaCl_2$ and $MgCl_2$)	Gibco	14025-050	500 mL
Optiplate-384, White Opaque assay plate (1)	PerkinElmer Inc.	6007290	50/box
AlphaPlate-384, Light Gray Opaque assay plate (2)	PerkinElmer Inc.	6005350	50/box
TopSeal-A 384, clear adhesive sealing film	PerkinElmer Inc.	6050185	100/box
Veriti 96 Well Thermal Cycler : 96-well format PCR Machine, with 6 temperature zones (6 x 2 columns), useful for performing target melting curves experiments	Applied Biosystems	-	-
ProFlex PCR System : Can accommodate 2 x 384-well plates at once; with a single temperature all across the plate	Applied Biosystems	-	-
Microplate shaking table	-	-	-
Envision®, Ensight™ or Victor Nivo® Alpha-reader	PerkinElmer Inc.	-	-

(1) Plates used for the immunoassay; (2) Same as (1) but optimal if cross-talk needs to be reduced; For more assay plates options, please go to www.perkinelmer.com/microplates

Buffer Preparation and Subsequent Storage Conditions

2X Lysis Buffers	Dilute each 5X Lysis Buffer in MilliQ water to a final concentration of 2X. For example: for 5 mL of 2X Lysis Buffer, add: 2 mL of 5X Lysis Buffer to 3 mL MilliQ water. Excess 2X Lysis Buffer should be discarded.
Acceptor Mix (Reaction Buffer 1 + Reaction Buffer 2 + Activation Buffer + AlphaLISA® Capt*Sure*™Acceptor Beads)	Dilute Activation Buffer **25-fold** in combined Reaction Buffer 1 and Reaction Buffer 2. Dilute Acceptor Beads **50-fold** in combined Reaction Buffers. For example: for 300 µL of Acceptor Mix: Combine 141µL of Reaction Buffer 1 and 141µL of Reaction Buffer 2, and to this add 12µL Activation Buffer and 6µL Acceptor Beads. The Acceptor Mix should be made up and used within 30 minutes for best results. Excess Acceptor Mix should be discarded.
Donor Mix (Dilution Buffer + Alpha Streptavidin Donor Beads)	Dilute Donor Beads **50-fold** in Dilution Buffer. For example: for 300 µL of Donor Mix, add: 6 µL Donor Beads to 294 µL of Dilution Buffer. The Donor Mix should be made up and used within 30 minutes for best results. Prepare and use under low light conditions. Excess Donor Mix should be discarded.
Positive Control Lysate	Reconstitute with 250µL water. Store at -20°C in single use aliquots and use within 3 months. Dilute as required in 1X CETSA Cell Lysis Buffer.

Precautions

The Alpha Streptavidin Donor Beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor Beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco, or the equivalent) can be applied to light fixtures. The Donor Beads should NOT be used under red/orange light as can be found in photographic work darkrooms, as red light (680nm) excites the beads.

Alpha *SureFire*® CETSA® Assay Protocols

The most relevant CETSA® assay is performed on living cells, as data obtained in such protocols reflect the ability of the compound to reach and bind the target in a close-to-physiological situation.

However, there is a possibility to run CETSA® assays on cells disrupted by freeze-thawing prior to being placed in the presence of the test compounds. This may provide valuable information about the ability of the compound to engage the target, independently of its permeability and metabolization properties by a living cell.

A. Intact Cells CETSA® assay

Cell Preparation

1. Recover cells directly (suspension cells) or by trypsination (adherent cells) from T-Flasks, Petri Dishes or Cell Factories (or any other culture method in place for the cell type used). Then wash the cells in HBSS to remove trypsin and resuspend cells in HBSS. A common density to start with is 1 to 2 million cells/mL, but cell density is part of the initial optimization of CETSA® assays and this can vary from cell type to cell type and according to the target to be detected.

Cell Treatment

2. In a PCR plate, add 10 µL/well of 2X concentrated test compound diluted in HBSS. When performing concentration-response testing, it is recommended to do a first dilution in 100% DMSO and then further dilute each compound concentration in HBSS, in order to keep the final DMSO concentration the same in all samples.

   Note: DMSO concentration should be kept at maximum 0.1% final on cells in order to avoid toxic effects.

3. Add 10 µL/well of cells resuspended in HBSS.
4. Incubate at 37°C / 5% CO_2 (in a cell culture incubator) for 60 min.

   Note: 30 minutes is often sufficient for intracellular targets, but the optimal incubation time may vary according to the target and cell type used.

   Note: Some targets are unstable and incubation at lower temperatures than 37°C may be required.

5. Pre-heat the PCR thermocycler to the selected temperature.

   Note: For measuring a target melting curve, a thermocycler with variable temperature zones is very useful at this stage. - Please pay attention that “gradient PCR machines” do not provide a precise control of the temperature over different zones and therefore are not recommended for running melting curves as this could introduce a bias in the true melting temperature calculation. – A standard melting curve typically includes 12 different temperatures ranging between 37-63°C. When one temperature for CETSA® screening applications has been selected, a thermocycler with a single temperature zone can be used.

   Important Note: The heating of the cover should be inactivated, else this would result in higher than desired sample temperatures and inaccuracy of melting temperature calculation.

6. Transfer the plate into the PCR thermocycler and heat the samples for 3 minutes.

   Note: Heating time is an important parameter, and it is important to strictly control it. Using shorter or longer heating times may result in a different concentration-response profile. In particular, compounds with different retention times by the target (off-rates) are expected to be impacted differently by changes in plate heating time.

7. Remove the plate from the thermocycler and allow it to cool down to 4°C on ice or use the thermocycler for fastest possible cooling. Incubate for at least 3 minutes.

Lysate Preparation

8. Add 20 µL of freshly prepared 2X CETSA® Cell Lysis Buffer. Agitate on a plate shaking table (350 to 700 rpm) for 30 minutes at room temperature.

   Note: An additional lysis step of flash freezing in liquid nitrogen and thawing at 20°C using the thermocycler can be implemented at this stage if desired as it may in some cases improve the day-to-day and/or well-to-well signal variability.

   Note: make sure you comply with standard lab safety procedures when working with liquid nitrogen to avoid any burning by liquid nitrogen. In particular wear protective glasses and gloves.

9. Pipet 20 µL up and down three times to ensure homogeneity of the cell lysate solution.
10. Transfer 10 µL of the lysate to a 384-well Optiplate™ or AlphaPlate™ microplates for the immunoassay. Add 10 µL of Control Lysates samples to separate wells. We recommend testing a serial dilution of Control Lysate (e.g. 100, 50, 25, 12.5, 6.25 and 0% diluted in 1X CETSA Cell Lysis Buffer).

SureFire CETSA® Assay

11. Add 5 µL of Acceptor Mix to the wells. Seal plate with Topseal-A adhesive film and incubate for 1 hour at room temperature.
12. Add 5 µL of Donor Mix to the wells under subdued light. Seal plate with Topseal-A adhesive film, and protect the plate from light. Incubate for 1 hour at room temperature in the dark.

    Note: Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.

13. Read plate on an Alpha Technology-compatible plate reader, using standard Alpha settings.

Alpha *SureFire*® CETSA® flowchart - Intact Cells assay

1. Harvest and resuspend the cells to the desired cell density[1] in Hanks Balanced Salt Solution.

2. Add 10 µL compound diluted in HBSS to 2x final concentration in a 96 or 384 well PCR plate.

3. Add 10 µL cells at 2x final concentration to the 96 or 384 well PCR plate, containing compounds.

4. Incubate for 60 minutes at 37°C, with gentle continuous rotation.

5 & 6. Transfer the PCR plate to a pre-heated PCR thermocycler.

6. Heat challenge the cell-compound suspensions for 3 minutes[2].

7. Allow the PCR plate to cool down to 4°C on ice or use the thermocycler for fastest possible cooling.

8. Add 20 µL of 2x Cell Lysis Buffer and incubate for 30 minutes on a plate shaker (350 to 700 rpm).

9. Pipet 20 µL up and down 3 times to ensure homogeneity of cell lysate.

10. Transfer **10 µl** of lysate (or diluted positive control lysate, or Lysis Buffer alone) to a white 96 or 384 well detection plate.

Acceptor Mix:	Typical volume (60 x 5 µL)	My volumes
Reaction Buffer 1	141 µL	
Reaction Buffer 2	141 µL	
Activation Buffer	12 µL	
Acceptor Beads	6 µL	

11. Add **5 µl** of **Acceptor Mix.**

Seal the plate and shake 1-2 minutes on a plate shaker. Incubate ≥1 h (RT° or 22°C).

Donor Mix:	Typical volume (60 x 5 µL)	My volumes
Dilution Buffer	294 µL	
Donor Beads	6 µL	

12. Add **5 µl** of **Donor Mix** under subdued light.

Seal, protect from light and shake for 1-2 minutes on a plate shaker. Incubate ≥1 h, up to overnight (RT° or 22°C). Allow thermal equilibration to plate reader temperature prior to reading.

13. Read plate.

[1] A starting point is usually 1 to 2 million cells/mL, but optimal cell density will depend on the target and cell type

[2] A standard melting curve includes 12 different temperatures ranging between 37-63°C. Use a uniform temperature for single concentration or concentration-response compound screening.

B. Disrupted Cells CETSA® assay

Cell Preparation

1. Recover cells directly (suspension cells) or by trypsinization (adherent cells) from T-Flasks, Petri Dishes or Cell Factories (or any other culture method in place for the cell type used). Then wash the cells in HBSS to remove trypsin and resuspend cells in HBSS. A common density to start with is 1 to 2 million cells/mL, but cell density is part of the initial optimization of CETSA® assays and this can vary from cell type to cell type and according to the target to be detected.

Cell disruption

2. Dispense the cell suspension in 1 mL to 10 mL tubes.
3. Freeze the cells by plunging the tubes into liquid nitrogen, until the tube content is completely frozen.

   Note: make sure you comply with standard lab safety procedures when working with liquid nitrogen to avoid any burning by liquid nitrogen. In particular wear protective glasses and gloves and close the tubes tightly to avoid any risk of tube explosion consecutive to liquid nitrogen entering in the tubes.
4. Thaw cell suspensions by plunging the tubes in a water bath at 20°C, until the tube content is completely thawed.
5. Repeat the freeze thawing three times.
6. Optional: Centrifuge the tubes for 15 min at 20 000xg to remove cell debris and transfer the supernatant into a new tube, and use the disrupted cells suspension immediately

   Note 1: In some cases using non-centrifuged disrupted cell suspension can lead to elevated background. In such case, the suspension can be cleared by centrifugation, as this usually decreases the background signal.

   Note 2: Depending on the target and cell type used, there may be a possibility to store the disrupted cell solution at -80°C for later use. However, some targets may degrade over time when stored in such conditions, and this needs to be validated on a case-by-case basis.

Disrupted cells Treatment

7. In a PCR plate, add 10 µL/well of 2X concentrated test compound diluted in HBSS. When performing concentration-response testing, it is recommended to do a first dilution in 100% DMSO and then further dilute each compound concentration in HBSS, in order to keep the final DMSO concentration the same.
8. Add 10 µL/well of disrupted cell suspension prepared above.
9. Incubate at 37°C / 5% CO_2 (in a cell culture incubator) for 30 minutes.
10. Pre-heat the PCR thermocycler to the selected temperature.
11. Transfer the plate into the PCR thermocycler and heat the samples for 3 minutes.

    Note: Heating time is an important parameter, and it is important to strictly control it. Using shorter or longer heating times may result in a different concentration-response profile. In particular, compounds with different retention times by the target (off-rates) are expected to be impacted differently by changes in plate heating time.

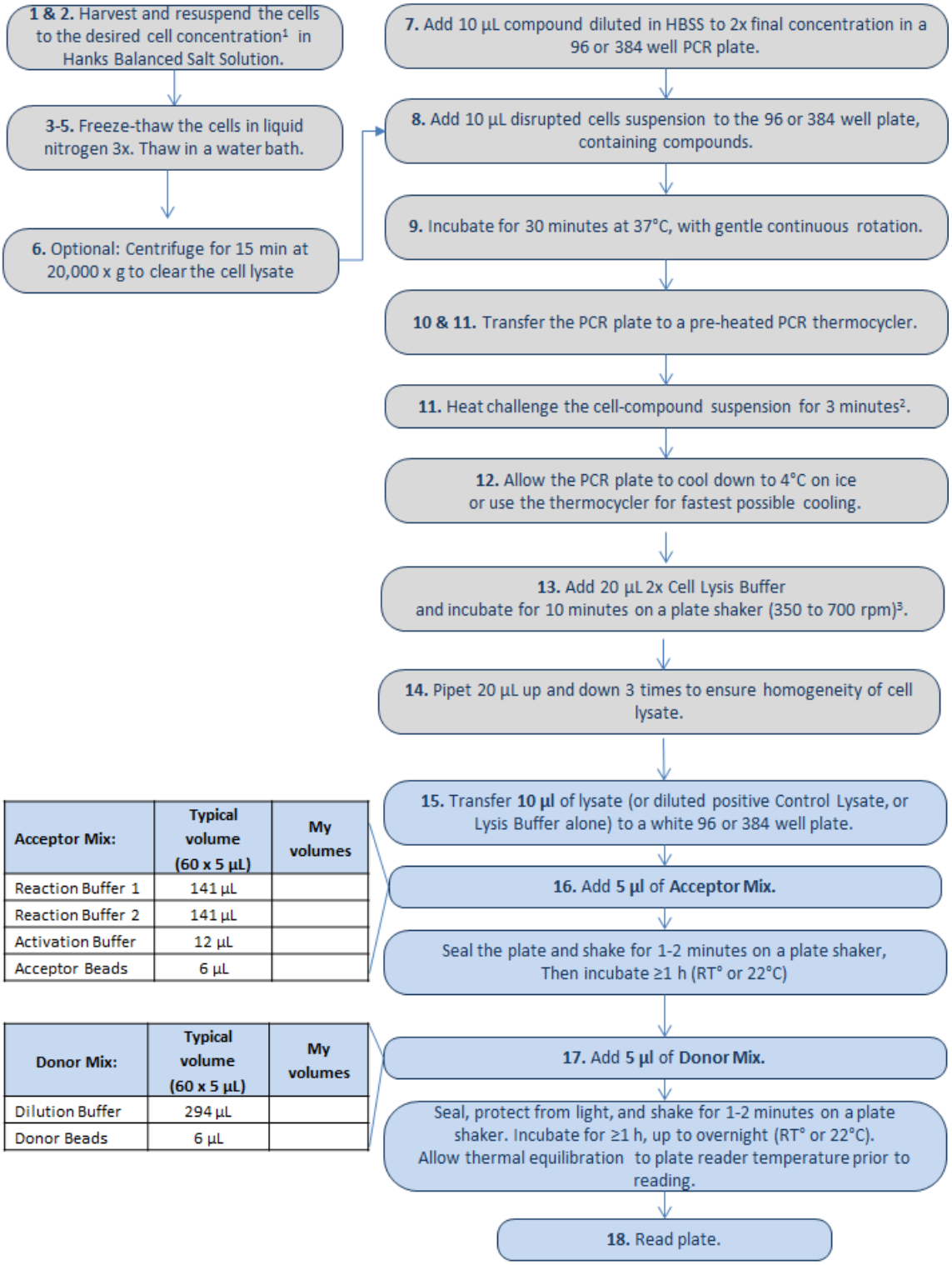
12. Remove the plate from the PCR thermocycler and allow it to cool down to 4°C on ice or use the thermocycler for fastest possible cooling. Incubate for at least 3 minutes.
13. To keep the same buffer conditions as when working with intact cells, add 20 µL of freshly prepared 2X CETSA Cell Lysis Buffer. Agitate on a plate shaking table (350 to 700 rpm) for 10 minutes at room temperature.
14. Pipet 20 µL up and down three times to ensure homogeneity of the cell lysate solution.
15. Transfer 10 µL of the lysate to a 384-well Optiplate™ or AlphaPlate™ for the immunoassay. Add 10 µL of Control Lysates samples to separate wells. We recommend testing a serial dilution of Control Lysate (e.g. 100, 50, 25, 12.5, 6.25 and 0% diluted in 1X CETSA Cell Lysis Buffer).

SureFire CETSA® Assay

16. Add 5 µL of Acceptor Mix to the wells. Seal plate with Topseal-A adhesive film, and incubate for 1 hour at room temperature.
17. Add 5 µL of Donor Mix to the wells under subdued light. Seal plate with Topseal-A adhesive film, and protect the plate from light. Incubate for 1 hour at room temperature in the dark.

    Note: Longer incubation may give greater sensitivity. Plates can be incubated overnight if required.
18. Read plate on an Alpha Technology-compatible plate reader, using standard Alpha settings.

Alpha *SureFire*® CETSA® flowchart - Disrupted Cells assay



Acceptor Mix:	Typical volume (60 x 5 µL)	My volumes
Reaction Buffer 1	141 µL	
Reaction Buffer 2	141 µL	
Activation Buffer	12 µL	
Acceptor Beads	6 µL	

Donor Mix:	Typical volume (60 x 5 µL)	My volumes
Dilution Buffer	294 µL	
Donor Beads	6 µL	

[1] A starting point is usually 1 to 2 million cells/mL, but optimal cell density will depend on the target and cell type.
[2] A standard melting curve includes 12 different temperatures ranging between 37-63°C. Use a uniform temperature for single concentration or concentration-response compound compound screening.
[3] For a comparative buffer conditions to an intact Cells Assay, the Lysis Buffer is added at this step.

Supplementary Buffers and Beads

If using the standard protocol, sufficient amounts of buffers and beads are provided in the kit. However if the standard protocol is modified, more buffers or beads may be needed. In this case, additional buffers and beads can be ordered using the following catalog numbers:

Item	Suggested source	Catalog #	Size
CETSA® Cell Lysis Buffer 1	PerkinElmer Inc.	CETSA-BUF1-100mL	100mL
CETSA® Cell Lysis Buffer 2	PerkinElmer Inc.	CETSA- BUF2-100mL	100mL
CETSA® Cell Lysis Buffer 3	PerkinElmer Inc.	CETSA- BUF3-100mL	100mL
CETSA® Cell Lysis Buffer 4	PerkinElmer Inc.	CETSA- BUF4-100mL	100mL
CETSA® Cell Lysis Buffer 5	PerkinElmer Inc.	CETSA- BUF5-100mL	100mL
Activation Buffer - *Ultra*	PerkinElmer Inc.	ALSU-AB-10mL	10mL
	PerkinElmer Inc.	ALSU-AB-100mL	100mL
Dilution Buffer - *Ultra*	PerkinElmer Inc.	ALSU-DB-10mL	10mL
	PerkinElmer Inc.	ALSU-DB-100mL	100mL
Alpha Streptavidin Donor Beads -2mg/mL	PerkinElmer Inc.	ALSU-ASDB-0.06mL	60µL
	PerkinElmer Inc.	ALSU-ASDB-1.2mL	1.2mL
	PerkinElmer Inc.	ALSU-ASDB-6mL	6mL
AlphaLISA® Capt*Sure*™ Acceptor Beads - 2mg/mL	PerkinElmer Inc.	ALSU-ACAB-0.06mL	60µL
	PerkinElmer Inc.	ALSU-ACAB-1.2mL	1.2mL
	PerkinElmer Inc.	ALSU-ACAB-6mL	6mL

Useful Links

For FAQ and troubleshooting, please go to:
www.perkinelmer.com/CETSAFAQ

For a complete list of Alpha *SureFire*® CETSA® kits, please go to:
http://www.perkinelmer.com/category/alpha-CETSA-kits or
www.tgrbio.com or
https://www.pelagobio.com/

For technical support please go to:
www.perkinelmer.com/ASK

Recommended CETSA® Reading

Martinez-Molina. D. (2013). Monitoring Drug Target Engagement in Cells and Tissues Using the Cellular Thermal Shift Assay. Science **341**:84-87.
https://science.sciencemag.org/content/341/6141/84

Seashore-Ludlow B, Axelsson H, Almqvist H, Dahlgren B, Jonsson M, Lundbäck T. (2018) Quantitative Interpretation of Intracellular Drug Binding and Kinetics Using the Cellular Thermal Shift Assay. Biochemistry **57**:6715-6725. https://dx.doi.org/10.1021/acs.biochem.8b01057

Shaw J, Dale I, Hemsley P, Leach L, Dekki N, Orme JP, Talbot V, Narvaez AJ, Bista M, Martinez-Molina D, Dabrowski M, Main MJ, Gianni D. (2019) Positioning High-Throughput CETSA in Early Drug Discovery through Screening against B-Raf and PARP1. SLAS Discovery **24**:121-132.
https://journals.sagepub.com/doi/10.1177/2472555218813332

More complete list of publications available at **www.pelagobio.com/Publications**