APPLICATION NOTE

DELFIA TRF

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Measuring Host Cell Protein Contamination in Recombinant Therapeutic Protein Production by DELFIA TRF as an Alternative to ELISA

Introduction

Therapeutic proteins (biologics) are produced in engineered host cell lines such as Chinese Hamster Ovary (CHO). Along with the expression of the protein of interest, host cells release additional proteins (Host Cell Proteins, HCPs) into the supernatant which must be removed from the drug product because they pose a risk of affecting drug quality, safety and efficacy.[1] HCPs are related to normal cell functions such as cell growth, proliferation, survival, gene transcription and protein synthesis. HCPs also arise as a result of cell apoptosis/death/lysis and constitute a complex mixture with diverse physiochemical and immunological properties.[2] The general acceptable limit of HCPs in biologics has been 1-100 ppm or 1-100 ng of HCP per mg of therapeutic protein.[3] The potential immunogenicity of biologics due to HCP contamination is a large risk, hence the need for accurate measurement to track the reduction of HCPs throughout the manufacturing process.

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Measurement of the level of HCPs present in the sample occurs in the early phase of protein production and continues through the middle and late stage of purification ending with the final drug product. Commercial enzyme-linked immunosorbent assays (ELISAs) with anti-HCP polyclonal antibodies raised against HCP mixtures (supernatants or cell lysates) have been used successfully in the production process. Due to the abundance of HCPs in the first phases of purification and the limited dynamic range of most ELISAs, samples must be diluted to a few different concentrations. This increases the workload as the total number of samples increases with each dilution. Dissociation-Enhanced Lanthanide Fluorescent Immunoassay time-resolved fluorescence (DELFIA® TRF) reduces some of the tedious dilutions and repeated testing of samples by providing a broader dynamic range for the assay, decreasing overall the number of wells tested, while maintaining the sensitivity compared to an ELISA.

DELFIA TRF assays are designed to detect the presence of a compound or biomolecule using fluorescent lanthanide chelate labeled reagents in a sandwich format (Figure 1). DELFIA TRF assays are compatible with a variety of plate readers, and, being a wash-based technology, with most sample types. The fluorescence decay time of the lanthanide chelate label is much longer than traditional fluorophores, allowing efficient use of temporal resolution for reduction of background auto-fluorescence. The large Stokes shift (difference, in wavelength, between excitation and emission maxima) and the narrow emission peak of the lanthanide chelate contribute to an increased signal-to-background ratio. The lanthanide chelate is dissociated with the addition of the enhancement solution and a new highly fluorescent chelate is formed into a protective micellar solution. DELFIA lanthanide chelates require this dissociation/enhancement step for fluorescence. DELFIA TRF provides the needed sensitivity, wide dynamic range, rapid assay optimization, stable signal, and high throughput capacity needed to measure HCPs through the biologics manufacturing process as an alternative to the traditional ELISA.

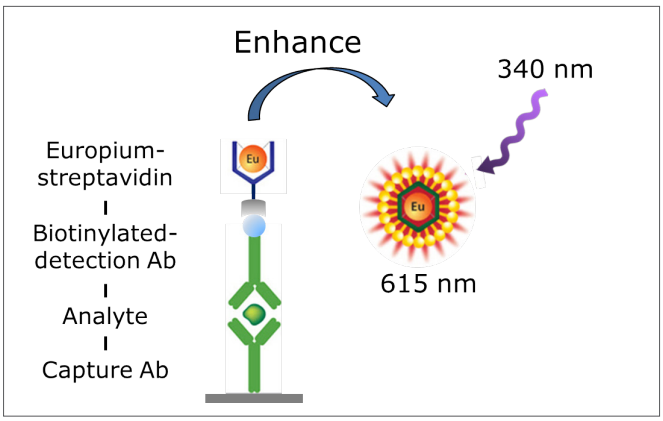

Figure 1. DELFIA TRF Europium-Streptavidin Assay. Direct assay configuration uses capture antibody directly adsorbed to a high bind DELFIA compatible plate. Biotinylated detection antibody recognizes bound analyte and the Europium labelled streptavidin binds to the biotin to complete the sandwich. Addition of enhancement solution releases the Europium from the complex to form a new highly fluorescent chelate that is measured on a plate reader using DELFIA TRF settings (excitation at 340 nm, emission at 615 nm).

Materials and Methods

Reagents

- DELFIA Streptavidin Toolbox Kit (PerkinElmer, #DFA100-HALF-5)
- Anti-CHO HCP antibody (Enzo Life Sciences, #ENZ-ABS260-0100)
- Anti-CHO HCP antibody, biotinylated (Enzo Life Sciences, #ENZ-ABS261-0100)
- CHO Host Cell Proteins analyte (Enzo Life Sciences, #ENZ-PRT122-0050)
- CHO Host Cell Proteins analyte (PerkinElmer, #AL301S)

Protocol

The DELFIA TRF workflow shown in Figure 2 is similar to the steps in a traditional ELISA. Whereas typical ELISA volumes require 100-150 µl of antibody and sample per well, DELFIA TRF can be easily miniaturized and performed in ½-AreaPlates requiring only 50 µL volumes which reduces assay cost by using less antibody and saves on potentially limited sample amounts.

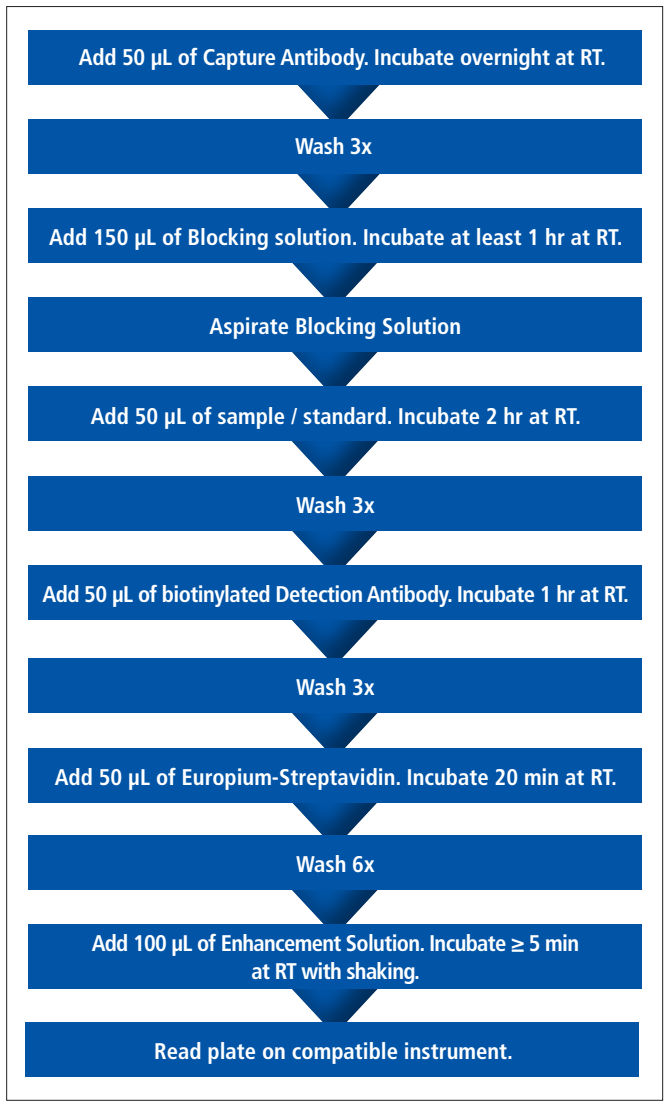

Figure 2. DELFIA TRF Workflow. Volumes listed are for the compact and convenient ½-AreaPlate format. Assay plates are coated with capture antibody prior to adding analyte and can be coated in bulk and stored in advance of running the assay similar to ELISA strip well plates. Total assay time is 5-6 hours and the assay workflow is amenable to automation.

Data Collection and Analysis

DELFIA TRF assay plates were read on an EnVision® 2105 multimode plate reader with stock settings for laser or lamp excitation of DELFIA TRF. Curves were plotted in GraphPad Prism® according to nonlinear regression fitting using the four-parameter logistic equation (sigmoidal dose-response curve with variable slope) and $1/Y^2$ data weighting. Data for the Enzo Life Sciences anti-CHO HCP ELISA (ENZ-KIT128-0001) was taken from the product manual and graphed for comparison to the DELFIA TRF anti-CHO HCP assay. ELISA sample data for the standard curve is representative of a routine run according to the manufacturer. Additional assay metrics for the Enzo Life Sciences ELISA are also reported in the product manual.

Results

Rapid, Easy Development of an Anti-CHO HCP DELFIA TRF Assay

The DELFIA Immunoassay Development Guide[4] was used to rapidly develop an assay to detect CHO HCPs using commercially available anti-CHO HCP antibodies and analyte from Enzo Life Sciences. Starting concentrations for capture and detection antibodies, as well as Europium-labeled streptavidin, are all listed in the guide. Minimal optimization was required to establish an assay that performed well by displaying good signal-to-background and relative sensitivity. Initial analyte concentration range was set larger than commercially available anti-CHO HCP ELISAs. As is the case with most DELFIA TRF assays, the dynamic range exceeds an ELISA for the same target with the increased range generally extending the higher concentrations on the curve. Figure 3 shows a titration of three concentrations of the biotinylated detection antibody at and below the level used in the Enzo Life Sciences anti-CHO HCP ELISA. The ideal concentration of detection antibody could be found empirically if no information from an existing ELISA is available.

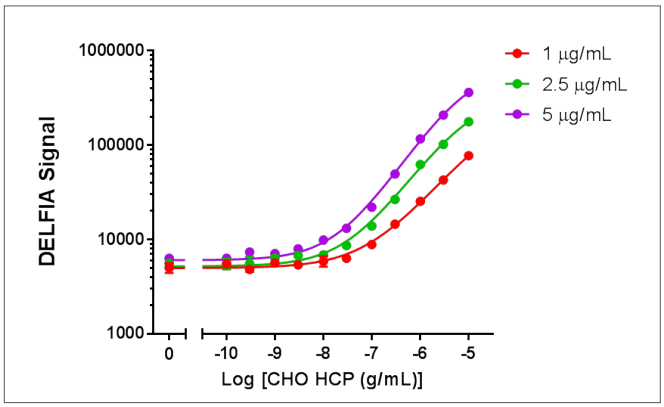

Figure 3. DELFIA TRF anti-CHO HCP Assay. Standard curves for DELFIA TRF were created using Enzo Life Sciences anti-CHO HCP antibodies and HCP analyte. 5 µg/mL of capture antibody was coated to the plates overnight. Three concentrations of biotinylated detection antibody were tested. 5 µg/mL of biotinylated detection antibody is used in the Enzo Life Sciences ELISA and provides the largest signal-to-background in DELFIA TRF (57) compared to the 2.5 µg/mL (32) or 1 µg/mL (15) concentrations. Data shown is from an EnVision 2105 using the flash lamp as the excitation source.

Comparison of DELFIA TRF to Enzo Life Sciences Anti-CHO HCP ELISA

Standard curve data for the Enzo Life Sciences anti-CHO HCP ELISA was taken from the product manual and is representative of a routine run of the ELISA according to the manufacturer. Results were plotted in Figure 4 for comparison to the DELFIA TRF anti-CHO HCP assay. Figure 4 highlights the extended dynamic range of DELFIA TRF with more concentrations at the top of the curve when compared to the ELISA standard curve. The ELISA format is absorbance based and is hindered by the upper optical density (OD) limit at higher concentrations which require sample dilution for testing. Table 1 compares the assay metrics of the DELFIA TRF anti-CHO HCP assay to the Enzo Life Sciences anti-CHO HCP ELISA data from the product manual.

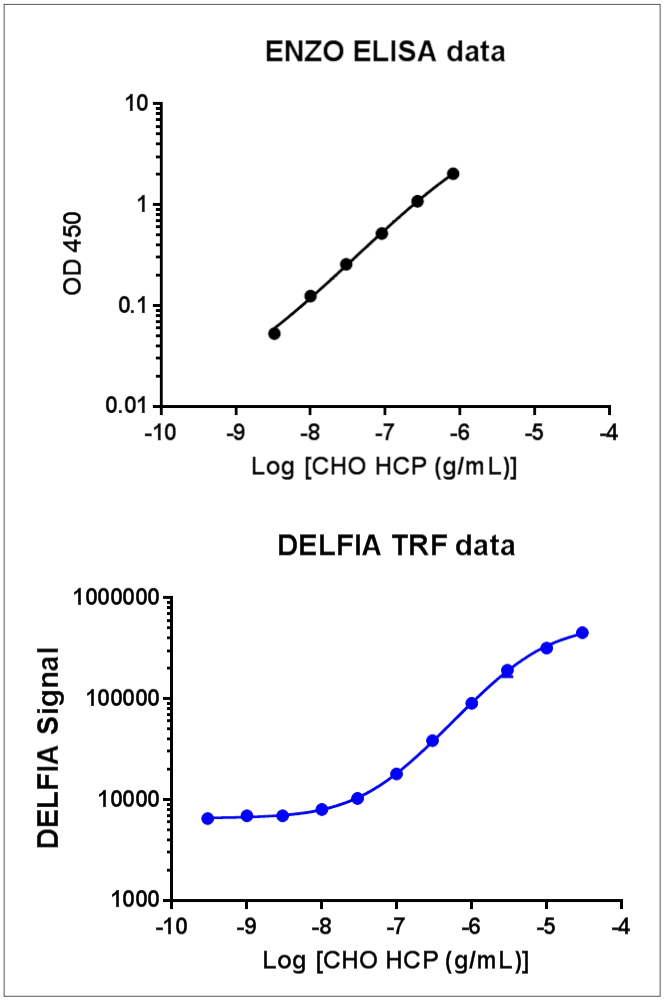

Figure 4. Enzo Life Sciences ELISA vs DELFIA TRF. Data for the standard curve was taken from the Enzo Life Sciences anti-CHO HCP ELISA product manual for comparison to the DELFIA TRF anti-CHO HCP assay. DELFIA TRF data for the analyte curve was taken from a representative experiment collected on an EnVision 2105 using the flash lamp as the excitation source. Results are plotted on the same x-axis scale to show the wider dynamic range of the DELFIA TRF assay.

Table 1. Assay Metrics Enzo Life Sciences ELISA Compared to DELFIA TRF. ELISA data is reported from the anti-CHO HCP ELISA product manual. Experiment derived values are listed for the DELFIA TRF from a representative assay run. In all parameters shown the DELFIA TRF anti-CHO HCP assay performs well compared to the ELISA with equal sensitivity (lower limit of detection = LDL), greater dynamic range, and comparable %CV (average value from multiple assay runs).

	Assay Range	LDL	Dynamic Range	%CV
ELISA (Enzo)	3 – 810 ng/mL	10 ng/mL	~ 2.5 log	4.5
DELFIA TRF	0.3 – 30000 ng/mL	8.5 ng/mL	~ 4 log	4.8

Spike and Recovery

Spike and Recovery was performed with two separate CHO HCP sources. The first source was the Enzo Life Sciences sample used to generate the standard curve (Table 2. S1-S3) and the second was from PerkinElmer (Table 2. S4-S5). Concentrations of analyte were spiked into DELFIA Assay Buffer and measured against the standard curve. Observed values were divided by the expected concentration and multiplied by 100 to reach the % Recovery. Typically passing recovery values fall within 70-130% of the expected concentration. All concentrations tested from both CHO HCP analyte sources passed.

Table 2. Spike and Recovery in the DELFIA TRF anti-CHO HCP Assay. Five different concentrations from two analyte sources were prepared in DELFIA Assay Buffer. S1-S3 are from the Enzo Life Sciences CHO HCP stock and S4-S5 are from the PerkinElmer CHO HCP stock. Percent Recovery was calculated as (Observed/Expected) x 100. All samples tested fell within the accepted 70-130% range for spike and recovery.

	Observed (ng/mL)	Expected (ng/mL)	% Recovery
S1 5000	4380.0	5000	87.6
S2 500	487.4	500	97.5
S3 50	42.9	50	85.7
S4 1000	998.4	1000	99.8
S5 100	110	100	110.0

Summary

A DELFIA TRF assay for detection of CHO host cell proteins was rapidly developed using commercially available antibodies and analyte by following the DELFIA Immunoassay Development Guide.[4] Minimal optimization experiments were required to adjust the standard curve range and antibody concentration for capture and detection. All other aspects of the assay followed the stock workflow (Figure 2) for the ½-AreaPlate size assay. In this DELFIA TRF anti-CHO HCP assay, a starting concentration of biotinylated detection antibody used in the assay was available from the corresponding ELISA kit of the manufacturer allowing for an easy conversion to DELFIA TRF; however a titration was tested to confirm the optimal concentration (Figure 3). When not performing a straightforward ELISA conversion, the concentration of the detection antibody can be determined empirically through a similar testing procedure.

The DELFIA TRF anti-CHO HCP assay generated using the Enzo Life Sciences polyclonal antibody showed superior assay metrics when compared to the published Enzo Life Sciences anti-CHO HCP ELISA data (Figure 4, Table 1). The wider dynamic range of the DELFIA TRF assay will allow the user to run fewer dilutions of upstream bioprocess samples that have high levels of HCPs, resulting in a reduced workload when compared with the ELISA format. With a reduction in the number of wells allocated to dilutional linearity of the sample, the total number of wells tested in each run will also be reduced. This will save on reagent cost, such as antibody cost, and potentially reduce the total number of assay plates run at each phase of HCP testing starting from the purification steps to the drug product.

The DELFIA TRF workflow is also highly amenable to automation which increases the capacity of testing. The DELFIA signal after adding the enhancement solution is stable for a long period of time removing the automation bottleneck effect associated with an ELISA readout which has a limited time frame to collect data before the color fades away. All these added benefits taken together show that DELFIA TRF provides a first-rate alternative assay format to the traditional ELISA currently in use for host cell protein measurement during the manufacture of biologics.

References

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2. F. Wang, D. Richardson, and M. Shameem, (2015). Host-cell Protein Measurement and Control. *BioPharm International,* 28 (6): 32-38.
3. Zhu-Shimoni J et al, (2014). Host Cell Protein Testing by ELISAs and the Use of Orthogonal Methods. *Biotechnology and Bioengineering,* 111 (12): 2367-2379.
4. PerkinElmer DELFIA Immunoassay Development Guide (2019).

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