

**21-September-2023**

**AB000137C (SP 2/0 HCP ELISA Kit)**

**SP 2/0 Host Cell Protein ELISA Protocol**

For the determination of SP 2/0 Host Cell Proteins in bulk products expressed in SP 2/0 expression systems.

**Please read this protocol completely prior to performing the assay.**

**Assay Principle**

The SP 2/0 HCP ELISA kit is designed to quantitatively measure SP 2/0 HCP contamination in bulk products expressed in SP 2/0 expression systems. Please read the complete kit insert before performing this assay. A series of SP 2/0 HCP standards is prepared to generate a standard curve for the assay and all unknown sample concentrations should be read off this standard curve. SP 2/0 HCP standards or diluted unknown samples (suggested mAb concentration for testing is 4 mg/ml) are pipetted into the provided 96-well plate which has been pre-coated with polyclonal anti-SP 2/0 HCP antibodies (Capturing antibody) to capture SP 2/0 HCP from biologics samples. Following an incubation to allow capture of the SP 2/0 HCP by the antibodies on the plate, a second biotinylated polyclonal anti- SP 2/0 HCP antibody (Reporting antibody), conjugated with biotin, is added and incubated to allow it to bind to the captured SP 2/0 HCPs. After 45 min. incubation, the plate is washed and a Streptavidin-HRP (Horse Radish Peroxidase) conjugate is added and incubated for 30 minutes. The Streptavidin-HRP conjugate will be captured by any biotin labeled antibody bound to the plate. Following a wash step to remove unbound conjugate, TMB substrate is added and is converted by the captured HRP to a colored product in proportion to the amount of HCP bound to the plate. After a short incubation to allow color development, the reaction is stopped, and the intensity of the generated color is detected in a spectrophotometer plate reader capable of measuring 450 nm wavelength. A standard curve will be generated from the SP 2/0 HCP standards and used to calculate the concentration of SP 2/0 HCP in the unknown samples, after making suitable correction for the dilution of the sample.

**Materials and Samples Preparation**

**PBS**

Phosphate-buffered saline, pH 7.4.

**PBS-T**

Phosphate-buffer saline with 0.1% Tween-20.

**Blocking Buffer**

1% BSA in PBS-T.

**Coating Plate**

A clear plastic microtiter plate is needed to coat with polyclonal (Capturing) anti- SP 2/0 HCP IgG.

Recommend using Corning microplate with high binding capability (for example, Corning Costar Assay Plate, 96 well, catalog number: 9018).

**SP 2/0 HCP Protein Standards**

Take out 8 siliconized Eppendorf centrifugation tube (1.7 ml), mark the tubes as 810 ng/ml, 270 ng/ml, 90 ng/ml, 30 ng/ml, 10 ng/ml, 3.3 ng/ml, 1.1 ng/ml and 0 ng/ml respectively. Add 1,192 µl of blocking solution to the vial marked 810 ng/ml, add 800 µl of blocking buffer to each of the rest vials.

SP 2/0 HCP protein stock at 40 µg/ml in 1% BSA in PBS-T is stock-A (Stock-A is prepared properly from a more concentrated HCP standard stock such as 4 mg/mL). Take 24.3 µl of Stock-A add to 1176 µl of blocking buffer in the tube marked 810 ng/ml, mix by inverting the vial a few times. Take 400 µl dilute to the vial marked 270 ng/ml for a 1:3 dilution, repeat the process to dilute down to the 1.1 ng/ml vial. Take 400 µl from the 1 ng/ml and discard. Now all the vials should have 800 µl solution.

**Reporting antibody**

A biotin labeled polyclonal antibody will be diluted into 12 ml of Reporting Antibody buffer (5% BSA in 2xPBS-T, this is to reduce the non-specific binding of the reporting antibody) to give a 2.5 μg/ml working stock.

**Streptavidin-HRP Conjugate**

A Streptavidin – Horse Radish Peroxidase conjugate (for example the product from Thermo Fisher/Pierce Catalog Number: 21126) will be used. Immediately prior to the assay, dilute the HRP conjugate prepared in a stock solution of 1 mg/ml to give a 0.1 μg/ml working stock.

**TMB Substrate**

Use directly without dilution (can be purchased from Thermo Scientific or Array Bridge).

**Stop Solution**

A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.

**Plate Sealer**

One.

**Other Materials Required**

Distilled or deionized water.

Single- and multi-channel micro-pipettes with disposable tips to accurately dispense volumes 5-250 μL.

Plastic tubes (i.e. 1.5 ml) for sample dilution

Reagent reservoirs for sample addition

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4-parameter) fitting. Contact your plate reader manufacturer for details.

**Precautions**

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete protocol should be read and understood before attempting to use the product.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

**Procedural Notes**

Allow diluted reagents and buffers to reach room temperature (18-25°C) prior to starting the assay. Once the assay has been started, all steps should be completed in sequence and without interruption. You do not want the plate to dry out in between steps as this can cause high backgrounds or erroneous results. Make sure that required reagents and buffers are ready when needed. Prior to adding to the plate, reagents should be mixed gently (not vortexed) by swirling.

Avoid contamination of reagents, pipette tips and wells. Use new disposable tips and reservoirs, do not return unused reagent to the stock bottles/vials and do not mix caps of stock solutions.

Incubation time can affect results. All wells should be handled in the same order for each step.

Microplate washing is important and can affect results by giving erroneous results or high backgrounds. We recommend a multichannel pipette or use wash station to add 250 µl of buffer to each well across the plate, followed by a dumping out of contents (to a sink or other receptacle) with a rapid wrist motion. The plate should then be tapped firmly on a paper towel to shake out any remaining liquid. Avoid prolonged incubation is wash buffer when performing wash steps.

When making additions to the plate, be careful to avoid damaging the coating, for example by scratching the bottoms or the sides of the wells. One technique to avoid this is to make additions (for a right-handed person) from left to right across the plate, supporting the pipette tips on the right edge of the well with each addition and thus avoiding contact with the bottom or sides of the wells.

During the incubation times, the plate should be covered to minimize evaporation from the wells. This can be done with the adhesive covers provided or by stacking an empty plate on top.

After the last wash step and prior to adding the TMB substrate, wipe the bottom of the plate with a clean paper towel to ensure that moisture or fingerprints do not interfere with the OD reading.

Once the TMB substrate is added it will be converted by the captured HRP to a blue colored product. Generally, we find that a 12 minutes incubation is sufficient for enough color development to discern differences between the standards and the reaction should be stopped at this point. Bear in mind that, given sufficient time, even a small amount HRP is capable of converting all the TMB to product and if this happens it will be difficult to discern differences between differing concentrations of HCP. Keeping OD450 values well below 2.0 will result in greatest accuracy as at high absorbance values very little light is reaching the detector and measurements are error prone. (Remember that at an OD of 1.0 only 10% of the light is being detected and at an OD of 2.0 only 1% of the light is reaching the detector).

**Assay Protocol**

1. Use the plate layout sheet on the back page to plan sample layout on plate and also aid in proper sample and standard identification after the assay. We recommend that assays are carried out in duplicate or (preferably) triplicate in order to minimize spurious results.
2. Dilute your mAb sample in 1x blocking buffer to make it 4 mg/mL (this can be adjusted based on spiking studies, typically it is from 2 mg/mL to 8 mg/mL). Take 1 mL of the 4 mg/mL mAb solution and spike with 5 µL of SP 2/0 HCP prepared at 4 µg/mL to make a 20 ng/mL HCP spike. Pipette 100 μL of samples (mAb solution with and without spike, each has 6 replicates, column 7,8,9) or SP 2/0 HCP standards (each concentration has triplicates, column 4, 5, 6) into wells in the plate as outlined. Leave several wells empty for background binding determination. Cover plate and incubate plate 90 min. at room temperature.
3. During the above incubation, dilute the reporting antibody to 2.5 µg/ml in 12 ml of Reporting Antibody Buffer (5% BSA in 2x PBS-T).
4. Wash plate by emptying contents and adding 250 μL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
5. Pipette 100 μL of Reporting Antibody into each well. Cover plate and incubate plate 45 min. at room temperature.
6. During the above incubation, dilute the Streptavidin-HRP conjugate to 0.1 µg/ml in 12 ml of 1x Blocking Buffer.
7. Wash plate by emptying contents and adding 250 μL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
8. Pipette 100 μL of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 30 min. at room temperature.
9. Wash plate by emptying contents and adding 250 μL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
10. Add 100 μL of TMB substrate to each well. Monitor color development and stop reaction by adding 100 μL of Stop Solution to each well when color development within standards is sufficient. Generally, 12 minutes time will be sufficient.
11. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
12. Either graph the results on log graph paper or use the plate reader’s built-in 4-parameter fit software capabilities to calculate HCP concentration for each sample.

**Calculation of Results**

Average the triplicate OD readings for each standard, sample and background wells to give a mean OD reading. Subtract the averaged background values from the mean OD values to give a net OD value and create a standard curve using either log graph paper of 4-parameter fit software. Match OD values for the unknowns to [HCP] using the standard curve, remembering to correct for dilution.

**Typical data**

|  |  |  |
| --- | --- | --- |
| **Sample** | **Mean OD** | **[HCP]** |
| 1.1 ng/ml HCP | 0.422 |  |
| 3.3 ng/ml HCP | 0.409 |  |
| 10 ng/ml HCP | 0.433 |  |
| 30 ng/ml HCP | 0.502 |  |
| 90 ng/ml HCP | 0.650 |  |
| 270ng/ml HCP | 1.029 |  |
| 810ng/ml HCP | 1.723 |  |
| Unknown 1 |  |  |
| Unknown 2 |  |  |
| Unknown 3 |  |  |



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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **H** | **G** | **F** | **E** | **D** | **C** | **B** | **A** |  |
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|  |  |  |  |  |  |  |  | **12** |

**Plate Template**