CANOPY
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RESIDUAL PROTEIN A ELISA KIT

Complete kit for the determination of residual Protein A contamination bulk products purified by Protein A affinity processes.

INCLUDED

- Coated 96-Well Strip Plate
- Protein A Protein Standard
- 5x Dilution Buffer
- 10x PBS-T
- Reporting Antibody
- Streptavidin-HRP Conjugate
- TMB Substrate
- Stop Solution
- Plate Sealer

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PRODUCT MANUAL HCP-004

RESIDUAL PROTEIN A ELISA KIT

ASSAY PRINCIPLE

The Protein A ELISA kit is designed to quantitatively measure residual Protein A contamination in bulk products after Protein A purification processes. Please read the complete kit insert before performing this assay. The assay utilizes an assay plate which has been pre-coated with rabbit anti-Protein A antibodies. The test samples along with the control samples are pre-treated by heat denaturation to remove IgG and IgG-like proteins. It is important to remove IgG proteins prior to performing this assay as Protein A – IgG complexes (which are very stable) can prevent recognition of Protein A by the capture antibody on the plate. After the immobilized antibody captures residual Protein A present in the samples, the captured Protein A or Protein A like proteins is detected by biotin labeled detection antibody, followed by addition of Streptavidin peroxidase conjugate. This conjugate binds strongly to the biotin molecule on the detection antibody. The level of bound peroxidase conjugate present in each assay well is determined by addition of a Peroxidase substrate (TMB), which is acted upon by the peroxidase enzyme to generate a blue coloration. After a short incubation (10-15 minutes), the reaction is stopped by the addition of acid solution that generates a yellow coloration. The absorbance of each well in the assay plate at 450 nm is then recorded using a suitable plate reader. A series of Protein A standards are provided to generate a standard curve for the assay and all unknown sample concentrations should be read off this standard curve. It is not necessary to boil these standards as they are already free of contamination IgG molecules.

Note: sodium azide will interfere with this assay and should not be used in samples or buffers.

SUPPLIED COMPONENTS

ENTIRE KIT MUST BE STORED AT 4°C.

Clear 96-Well Strip Plate
Clear plastic strip-well microtiter plate coated with rabbit anti-Protein A HCP IgG. Can be used as individual strips.

Protein A Protein Standard (25 ng/ml, 850 µl)
Concentrated Protein A proteins sufficient for generating a standard curve from 25 ng/ml to 0.011 ng/ml (using a 3-fold serial dilution) in 1x Protein A dilution buffer. Protein A standard is also used as a sample spike-in at 8 ng/ml.

5x Protein A Dilution Buffer (15 ml)
1x Protein A Dilution Buffer is used for dilution of Reporting Antibody and Streptavidin-HRP conjugate The 10 ml of 5x concentrate should be diluted to 50 ml with 40 ml of milliQ water to achieve 1x Protein A Dilution Buffer. Protein A Dilution Buffer should **NOT** be used to dilute the samples.

10x PBS-T (30 ml)
1x PBS-T is used for wash steps. The 25 ml of 10x concentrate should be diluted to 250 ml with 225 ml of milliQ water to achieve 1x PBS-T.

Reporting Antibody (200µl/tube)
A biotin labeled rabbit polyclonal antibody specific for Protein A proteins. Immediately prior to the assay, dilute 150 µl into 15 ml of 1x Protein A Dilution Buffer.

Streptavidin-HRP Conjugate (4 µg/ml, 420 µl/tube)
A Streptavidin–Horse Radish Peroxidase conjugate in a stabilizing solution. Immediately prior to the assay, dilute 375 µl into 15 ml of 1x Protein A Dilution Buffer to give a 0.1 µg/ml working stock.

TMB Substrate (15 ml)
Use directly without dilution.

Stop Solution (15 ml)
A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Plate Sealer

OTHER MATERIALS REQUIRED

milliQ water

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

1.5 ml eppendorf tubes

Reagent reservoirs for sample addition

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

ASSAY PROTOCOL

1. Dilute the 10x PBS-T and 5x Protein A Dilution Buffer to 1x-strength with milliQ water. The 25 ml of 10x PBS-T should be diluted to 250 ml with 225 ml milliQ water. 10 ml of 5x Protein A Dilution Buffer should be diluted to 50 ml with 40 ml of milliQ water.

2. Prepare Protein A Standards by numbering eight 1.5 ml tubes and adding 600 μl of 1x Protein A Dilution Buffer to tubes 2-8. The first tube receives 300 μl of concentrated 25 ng/ml of Protein A Standard alone. Cap the 8th tube, this will be the blank. To tube 2, add 300 μl of the concentrated 25 ng/ml standard stock and mix well. Then serial dilute 300 μl of tube 2 across tubes 3-7 to generate the remainder of the standards. This will generate a standard curve of 25 ng/ml to 0 ng/ml with 3-fold dilutions. Pipette 100 μl of standards and blank onto the plate in triplicate.

3. Prepare test Samples: dilute test sample to 0.5 mg/mL protein concentration with PBS-T (Please note that the protein samples need to be diluted by PBS-T instead of the dilution solution). Based on the quantitation limit requirement, spike Protein A standard into the 0.5 mg/ ml protein sample for recovery testing. It is recommended to spike 125 μl of the 8 ng/ml protein A standard into 875 μl of the protein sample (please make sure the final protein concentration is 0.5 mg/ml and the total volume is 1 ml, smaller volume may impact protein precipitation and spike recovery) for a quantitation limit (QL) of 2 ng/mg (2 ppm, 1 ng/0.5 mg). Adjust levels of Protein A spike-in based on your QL requirement.

Denature and remove IgG from control (spiked) and test samples: place the samples in boiling water (about 95°C to 100°C) for **3.5 to 4.5 minutes, target is 4 minutes.** Allow tubes to cool at room temperature for at least **15 minutes, up to 2 hours**. Centrifuge at 10,000 to 12,000 rpm for 1 to 2 minutes to pellet protein, take the supernatant to perform the Protein A analysis. Cover plate with individual plate seal and incubate **1.5 hours** at room temperature.

4. During the above incubation, dilute the Reporting Antibody by adding 150 μl to 15 ml of 1x Protein A Dilution Buffer.

5. Wash plate by emptying contents and adding 250 μl of 1x PBS-T to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. **Repeat 1x PBS-T wash step two additional times.**

6. Pipette 100 μl of Reporting Antibody into each well. Cover plate with the plate seal and incubate plate **45 minutes** at room temperature.

7. During the above incubation, dilute the 4 μg/ml Streptavidin-HRP conjugate to 0.1 μg/ml by adding 375 μl to 15 ml of 1x Dilution Buffer.

8. Wash plate by emptying contents and adding 250 μl of 1x PBS-T to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. **Repeat 1x PBS-T wash step two additional times.**

9. Pipette 100 μl of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate **45 minutes** at room temperature.

10. Wash plate by emptying contents and adding 250 μl of 1x PBS-T to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. **Repeat 1x PBS-T wash step two additional times.**

11. Add 100 µl of TMB substrate to each well. Monitor color development. Generally 10 minutes will be sufficient; incubating longer may increase the background.

12. Stop reaction by adding 100 µl of Stop Solution to each well containing TMB when the color development within standards is sufficient.

13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. A standard curve should be generated from the Protein A protein standards and used to calculate the concentration of Protein A in the unknown samples, taking into account any unknown sample dilution made.