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## PICHIA HOST CELL PROTEIN ELISA KIT

Complete kit for the determination of Pichia host cell protein contamination in bulk products expressed in Pichia expression systems.

### KIT INCLUDES

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

## PRODUCT MANUAL HCP-003

# PICHIA HOST CELL PROTEIN ELISA KIT

## ASSAY PRINCIPLE

The Pichia Host Cell Protein (HCP) ELISA kit is designed to quantitatively measure host cell protein contamination in bulk products expressed in Pichia expression systems. Please read the complete kit insert before performing this assay. A series of Pichia protein standards are made from a provided stock solution and used to generate a standard curve for the assay and all unknown sample concentrations should be read off this standard curve. Pichia standards or diluted unknown samples are pipetted into the provided 96-well plate which has been precoated with anti-Pichia HCP antibodies to capture Pichia proteins from biologics samples. Following an incubation to allow capture of the Pichia protein by the antibodies on the plate, a second anti-Pichia HCP antibody, conjugated with biotin, is added and incubated to allow it to bind to the captured Pichia proteins. After a 45 minute 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 ten minute 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 Pichia protein standards and used to calculate the concentration of Pichia proteins in the unknown samples, after making suitable correction for the dilution of the sample.

# SUPPLIED COMPONENTS

ENTIRE KIT MUST BE STORED AT 4°C.

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## Clear 96-Well Strip Plate

Clear plastic strip-well microtiter plate coated with rabbit anti-Pichia HCP IgG. Can be used as individual strips.

## Pichia Protein Standard (2430 ng/ml, 600 µl)

Concentrated Pichia proteins sufficient for generating a standard curve from 810 ng/ml to 1.1 ng/ml.

## 5x Dilution Buffer (15 ml)

1x Dilution Buffer is used for dilution of Reporting Antibody and Streptavidin-HRP conjugate. 1x Dilution Buffer is used to dilute samples if necessary. The 15 ml of 5x concentrate should be diluted to 75 ml with 60 ml of milliQ water to achieve 1x Dilution Buffer.

## 10x PBS-T (30 ml)

1x PBS-T is used for wash steps. The 30 ml of 10x concentrate should be diluted to 300 ml with 270 ml of milliQ water to achieve 1x PBS-T.

## Reporting Antibody (1 mg/ml, 150 µl/tube)

A biotin labeled rabbit polyclonal antibody specific for Pichia cell proteins. Immediately prior to the assay, dilute the entire 150 µl into 15 ml of 1x Dilution Buffer to give a 10 µg/ml working stock.

## Streptavidin-HRP Conjugate (4 µg/ml, 375 µl/tube)

A Streptavidin-Horse Radish Peroxidase conjugate in a stabilizing solution. Immediately prior to the assay, dilute the entire 375 µl into 15 ml of 1x 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

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1. Dilute the 10x PBS-T and 5x Dilution Buffer to 1x-strength with milliQ water. Check both concentrate bottles for precipitates before proceeding and if found warm slightly in a water bath to dissolve before proceeding. The 30 ml of 10x PBS-T should be diluted to 300 ml with 270 ml milliQ water. 15 ml of 5x Dilution Buffer should be diluted to 75 ml with 60 ml of milliQ water.
2. Prepare the HCP standards by numbering eight 1.5 ml tubes and adding 1ml of Dilution Buffer to each. Cap the eighth tube, this will be the blank (0 ng/ml HCP). To tube one add 500 µl of the provided 2430 ng/ml HCP stock and mix well, this will be the 810 ng/ml standard. Then serially dilute 500 µl of tube one across tubes two through seven to generate the remainder of the standards, with an 8th tube serving as a blank using Dilution Buffer alone. Pipette 100 µl of each standard and the blank into the plate.
3. Pipette 100 µl of samples into wells in the plate. If necessary, first dilute the samples in 1x Dilution Buffer.
4. Cover plate with individual plate seal and incubate **1.5 hours** at room temperature.
5. During the above incubation, dilute the 1 mg/ml Reporting Antibody to 5 µg/ml by adding 75 µl to 15 ml of 1x Dilution Buffer.
6. 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.**
7. Pipette 100 µl of Reporting Antibody into each well. Cover plate with the plate seal and incubate plate **45 minutes** at room temperature.
8. During the above incubation, dilute the 4 µg/ml Streptavidin-HRP conjugate to 0.1 µg/ml by adding the entire 375 µl to 15 ml of 1x Dilution Buffer.
9. 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.**
10. Pipette 100 µl of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate **30 minutes** at room temperature.
11. 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.**
12. Add 100 µl of TMB substrate to each well. Monitor color development. Generally 10 minutes time will be sufficient; incubating longer may increase the background.
13. Stop reaction by adding 100 µl of Stop Solution to each well containing TMB when the color development within standards is sufficient.
14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. A 4-parameter fit standard curve should be generated from the Pichia protein standards and used to calculate the concentration of Pichia proteins in the unknown samples, taking into account any unknown sample dilution made.



# STANDARD CURVE

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Pichia Protein Standard Curve

