Pichia Yeast host cell protein (HCP) Residue ELISA Kit

Catalog Number. CSB-EQ33265PY

For the quantitative determination of Pichia Yeast(PY) host cell protein (HCP) concentrations in cell culture supernates, products produced during process of protein purification and final products.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: https://www.cusabio.com/

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for PY HCP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for PY HCP. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PY HCP bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

6.25 ng/ml-800 ng/ml.

SENSITIVITY

The minimum detectable dose of PY HCP is typically less than 1.56 ng/ml.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of PY HCP. No significant cross-reactivity or interference between PY HCP and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between PY HCP and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
High Concentration Standard (500 µg/ml)	1 x 50 µl
HRP-conjugate (100 x)	1 x 150 µl
Wash Buffer (100 x)	1 x 10 ml
Sample Diluent (10 x)	1 x 10 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to one week.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- 10 µL-1000 µL multichannel micropipette
- Sterilization deionized water or ultrapure water
- Pipettes and pipette tips.
- Sterilization EP tubes.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates Remove particulates by centrifugation for 15 minutes at 1000 x g (or 3000rpm) and take supernates for assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 1 day may be stored at 2-8°C, otherwise samples must be aliquoted and stored at -20°C (<3 months) or -80°C (<6 months) to avoid loss of bioactivity and contamination.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
5. Samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
6. Owing to the possible interference (e.g.,complex buffer solutions produced during the purification of target), the result may not be good.
7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature before use for 30 minutes.
- Deionized water or ultrapure water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. Sample Diluent

Centrifuge the vial before opening. Sample Diluent requires a 10-fold dilution. A suggested 10-fold dilution is 10 ml of Sample Diluent (10 x) + 90 ml of deionized water or ultrapure water, mix well.

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved.

2. HRP-conjugate

Centrifuge the vial before opening. HRP-conjugate (100 x) requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate (100 x) + 990 µl of Sample Diluent, mix well.

3. Wash Buffer

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 ml of Wash Buffer (100 x) into deionized water or ultrapure water to prepare 1000 ml of Wash Buffer, mix well.

4. Standard

Take 8 bottles for standard preparation, number: S0-S7, add 46 μl、490 μl、250 μl、250 μl、250 μl、250 μl、300 μl of **Sample Diluent** into corresponding tube.

Take 4 μl of High Concentration Standard (500 μg/ml) into first tube, shake well, the concentration of S0 is 40 μg/ml; then take 10 μl of S0 into second tube, shake well, the concentration of S1 is 800 ng/ml; then take 250 μl of S1 into third tube, shake well, the concentration of S2 is 400 ng/ml; then produced 2-fold dilution series until S6. Lastly pipette 100 μl of S6 into eighth tube, shake well, the concentration of S7 is 6.25 ng/ml. Mix each tube thoroughly before the next transfer. (All the process as below) .

Number	Sample Diluent	Add the solution into corresponding tube	Concentration
S0	46 μl	4 μl of High Concentration Standard	40 μg/ml
S1	490 μl	10 μl of S0	800 ng/ml
S2	250 μl	250 μl of S1	400 ng/ml
S3	250 μl	250 μl of S2	200 ng/ml
S4	250 μl	250 μl of S3	100 ng/ml
S5	250 μl	250 μl of S4	50 ng/ml
S6	250 μl	250 μl of S5	25 ng/ml
S7	300 μl	100 μl of S6	6.25 ng/ml

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a blank well with 50 µl of **Sample Diluent.**
4. Add 100 µl of **HRP-conjugate** and 50 µl of **Standard** or **Sample** per well. Standard need test in duplicate.
5. Mix well and then incubate for 1 hour at room temperature.
6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash buffer** (300 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 20 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15-30 minutes at room temperature. Protect from light.
8. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 20 second soak period following the addition of wash solution, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

毕赤酵母细胞宿主蛋白（HCP）残留酶联免疫试剂盒
使用说明书

【产品编号】CSB-EQ33265PY

【预期应用】ELISA 法定量测定细胞培养上清、蛋白纯化过程及终产物中毕赤酵母宿主细胞蛋白残留（HCP）残留。

【产品性能指标】

1、 检测范围：6.25ng/ml - 800ng/ml

2、 灵敏度：1.56ng/ml

3、 精密度：批内差 CV%<15%，批间差 CV%<15%

4、 特异性：本试剂盒特异性检测毕赤酵母宿主细胞蛋白（HCP）残留，且与其他相关蛋白无交叉反应。

【实验原理】

本试剂盒应用双抗体夹心法测定样本中毕赤酵母细胞宿主蛋白（HCP）残留水平。用纯化的毕赤酵母细胞宿主蛋白（HCP）抗体包被微孔板，制成固相抗体，往包被抗体的微孔中依次加入毕赤酵母细胞宿主蛋白（HCP），再与 HRP 标记的毕赤酵母细胞宿主蛋白（HCP）抗体结合，形成抗体-抗原-酶标抗体复合物，经过彻底洗涤后加底物 TMB 显色。TMB 在 HRP 酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的毕赤酵母细胞宿主蛋白（HCP）残留呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），通过标准曲线计算样品中毕赤酵母细胞宿主蛋白（HCP）浓度。

【试剂盒组成成分】

组份	装量
酶标板 (Assay plate)	96 孔
高浓度标准品 (High Concentration Standard)	1 x 50μl/瓶（500μg/ml）
辣根过氧化物酶标记抗体 (HRP-conjugate)	1 x 150μl/瓶（100×）
浓洗涤液 (Wash Buffer)	1 x 10ml/瓶（100×）
浓稀释液 (Sample Diluent)	1 x 10ml/瓶（10×）
底物溶液 (TMB Substrate)	1 x 10ml/瓶
终止液 (Stop Solution)	1 x 10ml/瓶
板贴	4

【存储条件及有效期】

1、 未开封的试剂盒避光保存于2-8℃。请在试剂盒标注的有效日期内使用。

2、 开封试剂盒最多可保存一周。

【所需试剂和器材】

标准规格酶标仪；高速离心机；灭菌 EP 管；容量瓶；

系列可调节移液器及吸头；多通道移液器；灭菌的去离子水或超纯水等

【样本采集及保存】

细胞培养物上清：标本1000 x g或3000rpm离心15分钟取上清，上清立即用于实验，或分装后于-20°C 或-80°C保存。避免反复冻融。

【试剂配制】

1、标准品

标记 8 只稀释管，分别标记 S0、S1、S2、S3、S4、S5、S6、S7，分别加入一定体积的样本稀释液：46μl、490μl、250μl、250μl、250μl、250μl、250μl、300μl。取高浓度标准品（500μg/ml）4μl 加入到 S0 管，轻轻吹打并颠倒混匀后，接着吸取 10μl 的 S0 到 S1 管，混匀后，取 250μl 的 S1 到 S2 管，依次以 2 倍比梯度稀释至 S6，最后取 100μl 的 S6 至 S7 管中。

标记	样本稀释液	加相应的试剂到不同标记的管中	浓度
S0	46μl	4μl高浓度标准品	40μg/ml
S1	490μl	10μl S0	800ng/ml
S2	250μl	250μl S1	400ng/ml
S3	250μl	250μl S2	200ng/ml
S4	250μl	250μl S3	100ng/ml
S5	250μl	250μl S4	50ng/ml
S6	250μl	250μl S5	25ng/ml
S7	300μl	100μl S6	6.25ng/ml

2、洗液工作液

浓洗涤液按1:100倍用去离子水或超纯水进行稀释。例如用量筒量取990ml去离子水或超纯水，倒入烧杯或其他洁净容器中，再量取10ml浓洗涤液，均匀加入，搅拌混匀，在临用前配妥。浓洗涤液有少许结晶，请将其置于室温，并轻轻震荡至晶体完全溶解。

3、样本稀释液

将浓稀释液按1:10倍用去离子水或超纯水进行稀释。例如用量筒量取90ml去离子水或超纯水，倒入烧杯或其他洁净容器中，再量取10ml浓稀释液，均匀加入，搅拌混匀，在临用前配妥。浓稀释液有少许结晶，请将其置于室温，并轻轻震荡至晶体完全溶解。

4、辣根过氧化物酶标记抗体工作液

辣根过氧化物酶标记抗体工作液按1:100用样本稀释液进行稀释。如将10μl辣根过氧化物酶标记抗体加入到990μl样本稀释液，混匀。

【重要提示】

1、 实验开始前，请准备好所有试剂。试剂或样本稀释时，均需混匀，混匀时尽量避免起泡。

2、 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便管盖和管壁上的液体集中到管底。

【操作步骤】

1、 将各种试剂移至室温平衡至少 30 分钟，按前述方法配制试剂，备用。

2、 加样：设置空白对照孔，加 50μl 样本稀释液。设标准品孔、待测样本孔，每孔各加辣根过氧化物酶标记抗体工作液 100μl，再分别加标准品或待测样本 50μl，轻轻晃动混匀，覆上板贴，室温温育 1 小时。

3、 弃去孔内液体，甩干，洗板 5 次。每次浸泡 20 秒，300μl/孔，甩干。

4、 依序每孔加底物溶液 100μl，室温避光显色 15-30 分钟。

5、 依序每孔加终止溶液 50μl，终止反应。

6、 在反应终止后 5 分钟内用酶标仪在 450nm 波长依序测量各孔的光密度（OD 值）。

【操作要点】

1、 为保证检测结果的准确性，建议标准品及样本均设双孔测定。每次检测均需做标准曲线。

2、 如标本中待测物质含量过高，请先用合适的溶液进行稀释，以使样本符合试剂盒的检测范围，最后计算时再乘以相应的稀释倍数。

3、 **加样**：加样时，请使用一次性的洁净吸头，避免交叉污染。加样时应尽量轻缓，避免起泡，将样本加于酶标板孔底部，切勿沿孔壁加样。一次加样时间最好控制在 10 分钟内，如标本数量多，推荐使用排枪加样。

4、 **温育**：为防止样本蒸发或污染，温育过程中酶标板必须覆上板贴，实验过程中酶标板应避

免处于干燥的状态。

5、 **洗涤**：洗涤过程非常重要，不充分的洗涤易造成假阳性。

(1) 手工洗板方法：吸去（不可触及孔壁和孔底）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下轻拍几次；将洗液工作液按 300μl/孔注入孔内，浸泡 20 秒。根据操作步骤中所述，重复此过程数次。

(2) 自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

6、 **显色**：为保证实验结果的准确性，底物反应时间到后应尽快加入终止液。可在加入底物溶液后每隔一段时间观察一下显色情况以控制反应时间（比如每隔 10 分钟）。当肉眼可见标准品前 3-4 孔有明显梯度蓝色，后 3-4 孔显色不明显时，即可加入终止液终止反应，此时蓝色立刻变为黄色。终止液的加入顺序应尽量与底物溶液的加入顺序相同。

7、 底物溶液应为浅蓝色或无色，如果颜色严重变深则必须弃用。底物溶液易受污染，请避光妥善保存。

【数据处理】

取标准品及样本值数值后绘制曲线，如果设置复孔，则应取其平均值计算。以标准品的浓度为横坐标，OD 值为纵坐标，在坐标纸上绘出标准曲线。推荐使用 4-PL 进行分析，计算出样本浓度。若样本检测前进行过稀释，最后计算时需乘以相应的稀释倍数，即为样本的实际浓度。

【说明】

1、 本试剂盒仅供研究使用。

2、 中、英文说明书可能会有不一致之处，请以英文说明书为准。

3、 不同批号试剂不能混用。不要用其它生产厂家的试剂替换试剂盒中的试剂。

4、 刚开启的酶标板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。

Notes