E. coli Host Cell Proteins (HCPs) ELISA Kit

96 Tests Kit
Enzyme Immunoassay for the Quantitative Detection of E. coli Host Cell Proteins (HCPs)
For Research and Manufacturing only
Catalogue No. JTC232-E. coli

Introduction

Recombinant protein expression in prokaryotic and eukaryotic host cells is now important for the production of biopharmaceutical products. In the prokaryotic system, Escherichia coli (E. coli) is the most commonly used host cell for the production of recombinant proteins. The advantages of using E. coli as a host expression organism are well known. The production and purification processes of these products have the potential to be impure by host cell proteins (HCPs). These impurities could reduce the therapeutic bio-drugs' effectiveness and cause toxic or immunological reactions. As a result, it is critical to keep HCP impurities as low as possible. JTC E. coli HCP kit has been developed based on enzyme-linked immunosorbent assay (ELISA) to detect HCP impurities in therapeutic substances and to evaluate HCP contaminations in biopharmaceutical products in order to assist manufacturers in controlling the amount of HCPs as much as possible.

Test principle

JTC E. coli HCP Kit is based on the principle of a sandwich solid phase ELISA. The assay system utilizes an anti-E. coli HCP polyclonal antibody for solid phase immobilization (on the wells) and an anti-E. coli HCP polyclonal antibody for the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwiched formation of E. coli HCP between solid phase and conjugated antibodies. After second wash step a solution of 3,3′,5,5′-Tetramethylbenzidine (TMB) is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped by adding of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. A 4-parameter logistic (4-PL) fit standard curve is used to calculate the concentration of HCPs in the sample. The concentration of E. coli HCP is proportional to the color intensity of the test sample in comparison with the standards.

Materials provided with the kit

1. Antibody coated wells (1×96-well plate), microtiter wells coated with polyclonal anti-E. coli HCP antibody
2. Assay buffer (1 vial, 6 mL), containing the solution to dilute samples, ready to use
3. Enzyme conjugate (1 vial, 12 mL), polyclonal anti-E. coli HCP antibody labeled with HRP in buffer, ready to use
4. Standards set (1 mL/vial), containing 0, 3, 6, 12, 25, 50 and 100 ng/mL of E. coli HCP, ready to use
5. Chromogen substrate reagent (1 vial, 12 mL), consisting of benzidine and hydrogen peroxide, ready to use solution
6. Wash solution (1 vial, 50 mL), contain Phosphate Buffered Saline solution with 0.05 % Tween 20 as detergent, concentrated (10×)
7. Stop solution (1 vial, 12 mL), 1 molar hydrochloric acid solution
8. Cardboard sealer (1 pcs)
9. Brochure Barcode

Materials required but not provided

- ELISA reader with 450 nm filter (if possible 630 nm as reference filter)
- Orbital rotator with a minimum speed of 200 rpm
- Precision pipettes: 50 µL and 100 µL
- Disposable pipette tips
- Distilled water
- Absorbent paper

Notes for consumers

1. The contents of the kit should be used only for the current kit.
2. All contents of the kit are for research use only.
3. Kit should be used only by qualified technicians.
4. Kit is designed and manufactured only for the measurement of E. coli HCP in biopharmaceutical products.
5. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
6. Kit is for Research or Manufacture Use Only.

Storage and stabilities

1. Kit should be stored at 2-8 °C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. For long term storage, in concentrated washing solution, crystals may form. Before preparing the work wash solution, place the vial at 37 °C to dissolve the crystals. Dilute the concentrated washing solution with distilled water, 1/10.

General information

1. Be sure to bring all reagents at room temperature 30 minutes before starting the test.
2. All steps should be performed non-stop from the start of the test. Do not allow the wells to dry between incubation stages.
3. Use a disposable sampler tip for each sample.
4. Strips should be read within 30 minutes after adding stop solution since color will fade over time.
5. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Precision on duplicate samples should yield coefficients of variation (CV) of less than 10% for samples. In cases, where the CV of two replicates of each sample is high, the plate washing process should be evaluated.
7. One of the most important factors in achieving the accurate result is the appropriate incubation time. Before starting the test, prepare the required materials and solutions, this will be improved the accuracy by decreasing the time interval between the sampling steps.
8. In samples with high concentrations of HCP, the linearity test is not observed in diluted samples. In these conditions, the minimum required dilution (MRD) must be calculated for the final or in-process production samples. A very important point is to use a compatible diluent to achieve accurate recovery.

Reagents preparation

1. Bring all reagents to reach room temperature (20-25 °C) before use.
2. Working wash solution: Dilute concentrated wash solution 1:10 with distilled water before use and store working wash solution at 2-8 °C.

Test procedure

1. Choose the appropriate number of coated wells and keep remaining wells in tightly closed special bag.
2. Add 50 µL of assay buffer into each well.
3. Dispense 50 µL of standards and samples into the wells in duplicate and shake wells gently for 15 seconds to mix well.
4. Cover the microtiter wells with cardboard sealer firmly. Incubate plate on orbital shaker at 200-600 rpm for 90 minutes at room temperature (20-25 °C).
5. Remove the sealer and take out wells contents by flicking the microplate into a waste container.
Rinse and flick the microtiter wells 5 times (each time with 300 µL of working wash solution). Strike the wells gently onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µL of anti- E. coli-HRP conjugate (enzyme conjugate) into the wells.
7. Seal the plate with cardboard sealer again and incubate plate on orbital shaker at 200-600 rpm for 30 minutes at room temperature (20-25 °C).
8. Repeat step 5.
9. Dispense 100 µL of chromogen/substrate (TMB) into the microplate wells.
10. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
11. Stop the reaction by adding 100 µL of stop solution to the microplate wells.

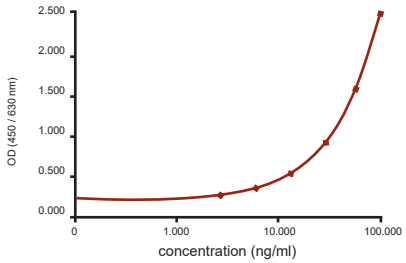
12. Measure absorbance at 450 nm by ELISA reader (use 630 nm filter as reference filter if it is available).

Result calculation

1. Calculate mean absorbance value of standards and samples at 450 nm (use 630 nm filter as reference filter if it is available).
2. Construct a 4-PL fit standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL using curve fitting software, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Calculate the corresponding concentration of E. coli HCP in the sample (ng/mL) from the standard curve using the mean absorbance value for each sample.

Example of E. coli HCP standard curve

Standards (ng/mL)	OD (450/630 nm)	Mean OD
0	0.043	0.041
	0.039	
3	0.108	0.108
	0.109	
6	0.163	0.175
	0.187	
12	0.351	0.352
	0.353	
25	0.698	0.704
	0.709	
50	1.305	1.30
	1.293	
100	2.491	2.483
	2.474	



Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

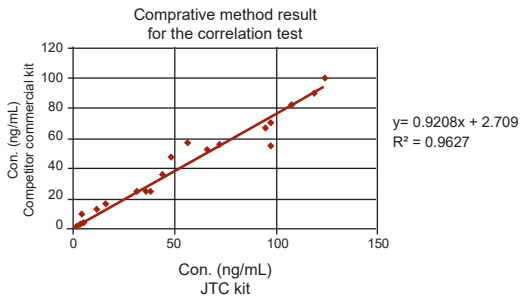
Limitations of the measurement method

1. Before using the kit to measure E. coli HCP, the quality of antibodies used in this kit and the test method should be evaluated by each laboratory in terms of specificity, accuracy and precision.
2. Despite designing the kit to avoid interfering with different agents. However, very excessive concentrations of some samples (often in the 2-5 mg/mL range) can also additionally interfere in the accurate measurement of HCPs.

Performance characteristics

1. Correlation test

E. coli HCP ELISA kit of JTC was compared with the relevant commercial kit. Twenty-three drug samples from different stages of production and purification were used for comparative tests. The results of comparative analysis on the samples showed a 96% correlation between JTC kit and a valid commercial kit.



2. Sensitivity

The lower limit of detection (LOD) is defined as that concentration corresponding to a signal three standard deviations above the mean of the zero standard. LOD is ~1.7 ng/mL.
The limit of quantitation (LOQ), is the lowest concentration at which coefficients of variation (CV) is less than 20% with acceptable accuracy. The LOQ is ~3.0 ng/mL.

3. Test precision

Both intra- (n=20 replicates) and inter- (n=20 replicates) assays precision was determined on three drug substance samples with different concentrations of E. coli HCP. Three drug substance samples with different concentrations of E. coli HCP were repeatedly tested. Results are shown in Tables 1 and 2:

Table 1. Intra-assay

Sample	No. of tests* performed	Means (ng/mL)	SD (ng/mL)	CV (%)
1	20	11.0	0.01	3.4
2	20	36.0	0.03	3.5
3	20	90.0	0.1	4.1

Table 2. Inter-assay

Sample	No. of tests* performed	Means (ng/mL)	SD (ng/mL)	CV (%)
1	20	11.0	0.02	6.5
2	20	36.0	0.08	7.6
3	20	90.0	0.11	4.2

*Each test has been run in duplicate

4. Hook effect

To rule out possible hook effect occurrence, the E. coli HCP assay was done on samples with high concentration of E. coli HCP (up to 100 µg/mL) and no "hook effect" was seen.

Schematic Procedure of E. coli HCP test		
Reagent	Standard	Sample
Assay Buffer	50 µL	50 µL
Standard	50 µL	-
Sample	-	50 µL
Shake the plate gently for 15 seconds and then cover the wells with cardboard sealer. Incubate for 90 minutes on the rotator at 200 rpm at RT. Remove the cardboard sealer of the plate and empty the contents of the wells. Wash the wells 5 times according to the washing instructions.		
Anti-E. coli HCP-HRP conjugate	100 µL	100 µL
Cover the wells with the cardboard sealer. Incubate for 30 minutes on a rotator at 200-600 rpm at RT. Remove the cardboard sealer from the plate and empty the contents of the wells. Wash the wells 5 times according to the washing instructions.		
TMB	100 µL	100 µL
Incubate wells for 15 minutes at RT in dark.		
Stop	100 µL	100 µL
Read absorbance at 450 nm (use 630 nm as reference filter if it is available).		

JTC Diagnosemittel GmbH (JTC Diagnostics)
Schulweg 7, D-34516 Voehl, Germany
Land Line Phone: +49 5635 9929364
Website: www.jtc-diagnostics.de
Email: thomas.nolte@jtc-diagnostics.de

District Court: Korbach
Commercial Register No.: HRB 1745
VAT No.: DE273871096
Tax No.: 025/236/80134
EORI No.: DE1145754