EIV32025E

ExCell Bio

ResiQuant® *E. coli* HCP ELISA Kit-Plasmid

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	CRH00-3041S
	CRH00-3041
	CRH00-3042

PRODUCT DESCRIPTION

Plasmids are circular double-stranded DNA molecules widely used in the fields of vaccine preparation and cell gene therapy (CGT), such as DNA vaccines, naked plasmid gene vectors, adeno-associated viruses (AAV), and lentiviral vectors. Plasmids are typically produced using *Escherichia coli (E. coli*) as a host, and the most representative host strains are derived from *Escherichia col*i K-12, such as DH5α, Stbl3, Top10, etc. During the production and purification of plasmids, there may be contamination from residual *Escherichia coli* host cell proteins (*E. coli* HCP). These residual HCPs can pose risks to the subsequent applications of the plasmids, such as triggering immune reactions in vivo. Therefore, it is important to control the level of residual *E. coli* HCP. Unlike the conventional process of expressing recombinant proteins, the most commonly used process for plasmid production and purification is alkaline lysis. The antigen used in this assay kit is denatured *E. coli* HCP prepared during the alkaline lysis process. Exclusive antibodies against alkaline lysis process-derived *E. coli* HCP were obtained through immunization of animals. This product is suitable for quantitative detection of *E. coli* HCP in plasmid intermediates, crude extracts, and final products using the alkaline lysis process.

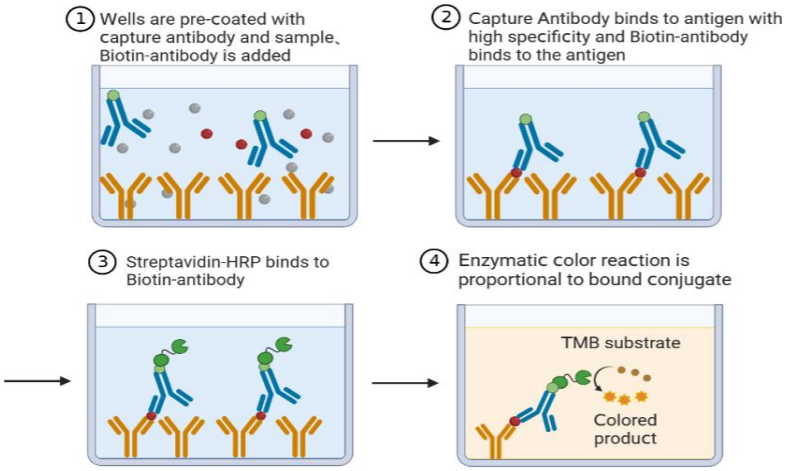
For the first time, it is recommended to conduct a suitability study to confirm the sample matrix effect and determine the appropriate dilution conditions for test.

PRINCIPLES OF THE TEST

The assay employs a sandwich immunoassay methodology for the quantitation of *E. coli* HCP. Microplates have been coated with antibodies specific for *E. coli* HCP. Subsequently, the standards or test samples and biotinylated anti-*E. coli* HCP antibodies are pipetted into the wells, standards or *E. coli* HCP in the samples form immunocomplex with immobilized antibody and biotinylated anti-*E. coli* HCP antibody. After a series of washes to remove unbound components, horseradish peroxidase (HRP)-conjugated streptavidin is added and bound to biotinylated anti *E. coli* HCP antibody. Following a further round of washes to eliminate unbound HRP-Antibody conjugates, the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) is introduced, initiating an enzymatic reaction. This reaction involves HRP-mediated oxidation of TMB in the presence of hydrogen peroxide (H_2O_2), yielding a blue-colored product that exhibits maximum absorbance at 655 nm. The enzymatic reaction is subsequently halted by the addition of a stop solution, which turns the color to yellow with

peak absorbance at 450 nm. The optical density (OD) value at 450 nm, measured by an enzyme-linked immunosorbent assay (ELISA) reader, is proportional to the concentration of *E. coli* HCP within the samples. Quantitative determination of *E. coli* HCP is achieved with a simultaneously prepared standard curve, using the standards supplied within the kit.

Schematic diagram:



PRODUCT PERFORMANCE

1. Sensitivity: The limit of detection (LOD) for *E. coli* HCP is 0.5 ng/mL, and the low limit of quantitation (LLOQ) is 1.0 ng/mL.
2. Precision: The coefficient of variation (CV) within the quantitation range is no more than 20% for both intra- and inter-assay.
3. Specificity: No cross-reactivity has been observed with CHO, Vero, HEK293 whole cell lysates.

PRODUCT APPLICATION

This product is a generic assay kit designed for the quantitative detection of *E. coli* HCP impurities in samples.

PRODUCT SPECIFICATIONS

Catalog Number	Name	Size
CRH00-3041S	ResiQuant® *E. coli* HCP ELISA Kit-Plasmid	48T
CRH00-3041	ResiQuant® *E. coli*i HCP ELISA Kit-Plasmid	48T
CRH00-3042	ResiQuant® *E. coli*i HCP ELISA Kit-Plasmid	96T

REAGENTS PROVIDED

Name	96 Tests	48 Tests	Store
***E. coli* HCP Standard**	3	2	2℃ to 8℃
***E. coli* HCP Microplate**	8× 12	8× 6	2℃ to 8℃
***E. coli* HCP 100× Biotin-Antibody**	60 µL	30 µL	2℃ to 8℃
100× Streptavidin-HRP	120 µL	60 µL	2℃ to 8℃
***E. coli* HCP Assay Diluent**	2× 25 mL	25 mL	2℃ to 8℃
20× Wash Buffer Concentrate	30 mL	30 mL	2℃ to 8℃
Substrate Solution	12 mL	6 mL	2℃ to 8℃ (Light-Sensitive)
Stop Solution	12 mL	12 mL	2℃ to 8℃
Plate Sealer	3	2	/

INSTRUCTION FOR USE

I. Materials required but not provided

1. Microplate reader capable of measuring absorbance at 450 nm (If wavelength correction is needed, additional wavelength at 630 nm or 570 nm is required).
2. 0.5-10 µL, 2-20 µL, 20-200 µL, 100-1000 µL adjustable micropipettes with disposable tips.
3. Microplate shaker.
4. Deionized water.

II. Sample collection

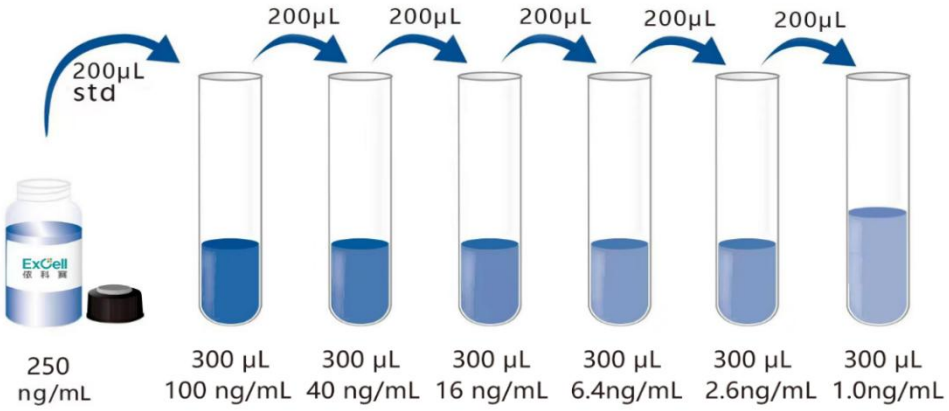
1. Samples should be clarified and any precipitate should be removed by centrifugation.

2. The sample should be diluted appropriately with *E. coli* HCP Assay Diluent according to the pre-determined condition (It is recommended to complete the applicability study to confirm the appropriate dilution conditions for sample detection).

III. Preparation of reagents

1. It is recommended to remove the assay kit from the refrigerator 20 minutes in advance to allow it to equilibrate to room temperature.
2. Dilute 20× Wash Buffer Concentrate with deionized or distilled water to prepare the wash buffer. Store the unused solution at 2℃ to 8℃.
3. Reconstitute the *E. coli* HCP standard with *E. coli* HCP Assay Diluent and dilute it to a concentration at 250 ng/mL. Mix thoroughly to ensure complete reconstitution and allow the standard to incubate for 15 minutes. A seven-point standard curve using 2.5-fold serial dilutions in the assay diluent which also serves as the zero standard (0 ng/mL). The following concentrations are recommended for standard curves:250,100, 40, 16, 6.4, 2.6, 1.0 and 0 ng/mL.

The dilution of standard solution:



Note: *Please aliquot the reconstituted stock solution (250 ng/mL) and store the unused stock solution at -18°C or lower in two months. Use freshly prepared diluted standards every time.*

4. Biotinylated Antibody Working Solution: Freshly prepare every time the working solution by diluting *E. coli* HCP 100× Biotin-Antibody with *E. coli* HCP Assay Diluent to 1× in an amount sufficient advance 30 minutes for the current experiment.
5. Enzyme Conjugate Working Solution: Freshly prepare every time the working solution by diluting 100× Streptavidin-HRP with *E. coli* HCP Assay Diluent to 1× in an amount sufficient advance 30 minutes for the current experiment.

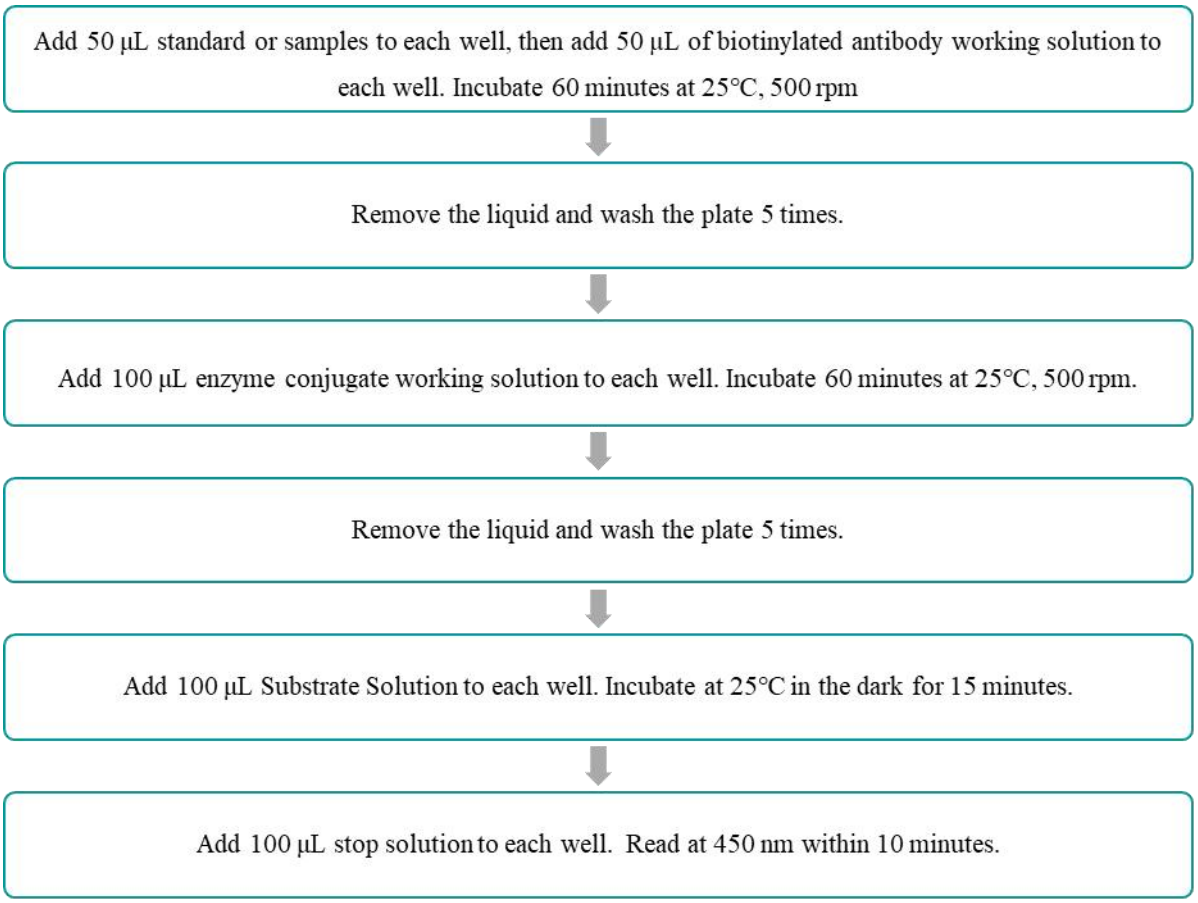
IV. Washing

1. Hand-wash: add 300 µL of wash buffer into each well, allow it to sit for 10 seconds, then pour off the liquid in the well. Invert the plate and blot it against clean paper towels. Wash the plate 5 times.
2. Automatic washing machine: set the volume to 300 µL, and the interval between aspiration and dispensing is 10 seconds. Wash the plate 5 times.

V. Assay procedure

1. Bring all reagents and samples to room temperature before use. Remove excess microplate strips from the plate frame, return them to the foil pouch containing a desiccant pack, reseal tightly and store at 2℃ to 8℃.
2. Set the blank well (If the plate is measured with dual-wavelength, the blank may not be necessary).
3. Prepare samples, standards and biotinylated antibody working solution in advance.
4. Add different concentrations of standards or samples to the microplate wells respectively, **50 µL** per well. , then add **50 µL** of biotinylated antibody working solution to each well. Seal the wells with the plate sealer. Incubate at **25℃** and shake for **60 minutes**, using a microplate shaker (500 rpm).
5. Prepare enzyme conjugate working solution in advance.
6. Remove the liquid from the wells and wash the plate 5 times.
7. Add **100 µL** enzyme conjugate working solution to each well. Seal the reaction wells with the plate sealer. Incubate at **25℃** and shake for **60 minutes**, using a microplate shaker (500 rpm).
8. Remove the liquid from the wells and wash the plate 5 times.
9. Add **100 µL** of Substrate Solution to the wells, Incubate at **25℃** in the dark for **15 minutes**.
10. Add **100 µL** of Stop Solution to the wells. Read the optical density (OD) of each well within 10 minutes using a microplate reader set to 450 nm. If wavelength correction is available, the OD value at additional wavelength (630 nm or 570 nm) should be collected. For single wavelength mode, the OD value of blank well shall be subtracted from that of each Standard or sample.

VI. Assay Procedure Summary

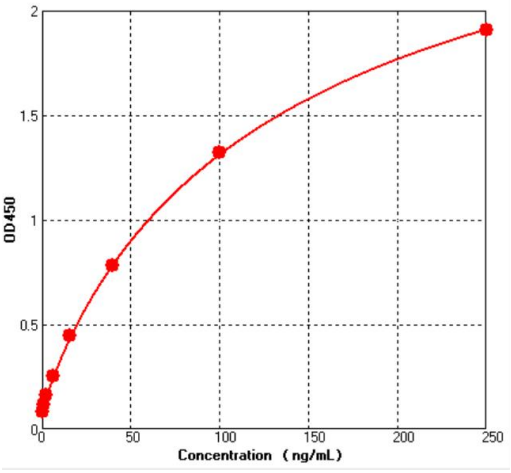


ANALYSES OF RESULTS

1. Create a standard curve by plotting the mean optical density (OD) of each standard concentration as the y-axis against the standard concentration on the x-axis. It is recommended to use a four-parameter fitting equation for the standard curve. Calculate the concentration of samples based on their OD values.
2. The coefficient of determination (R^2) for the standard curve should be no less than 0.99. Discard those abnormal data, the deviation between the back calculated and the theoretical concentration of each standard should be within ±20% (±25% for the upper or lower limits of quantitation).
3. If the OD value of the sample exceeds the high end of the standard curve, the sample should be appropriately diluted before further measurement. Be aware that the dilution factor should be counted with back calculated concentration together.
4. The concentration of samples should be calculated with the standard curve in the parallel assay.

Representative Standard Curve:

Standard (ng/mL)	**$OD_{450-630}$**			**Concentration (ng/mL)**		
	1	2	3	1	2	3
250	1.918	1.958	1.851	253.2	270.3	227.5
100	1.283	1.394	1.278	95.2	113.1	94.5
40	0.784	0.811	0.754	39.6	41.8	37.1
16	0.444	0.462	0.429	6.2	17.2	15.3
6.4	0.241	0.263	0.247	5.9	6.9	6.2
2.6	0.162	0.163	0.155	2.7	2.7	2.4
1.0	0.116	0.121	0.117	1.0	1.2	1.0
0	0.075	0.089	0.075	------	------	------
R^2	0.99982					



Note: *This graph is provided for reference purpose only. Data analysis was using ELISA Calc software with a four-parameter fitting model.*

NOTES

1. Store the reagents at 2℃ to 8℃. The reconstituted standard stock solution should be kept at -18℃ or below.
2. The concentrated Biotin-Antibody and Streptavidin-HRP are supplied in a small volume. Turbulence or inversion happened during transportation may cause spray of the solution elsewhere on the wall or the cap. Therefore, please spin the tubes shortly before use to harvest all liquid to the bottom of the tube.
3. In case crystal sediment appears in the 20× Wash Buffer Concentrate, warm it up at 37°C to dissolve the sediment before use.
4. Do not mix or substitute reagents with those from other lots or different kits.
5. Ensure thoroughly mixing in preparing the solutions to ensure the reactions are consistent.
6. It is recommended to test all samples and standards in duplicate form at least.

DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.
2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.