Elabscience®

Protein A HCP ELISA Kit

REF GEN002

TEST PRINCIPLE

These kits are intended for use in quantitating recombinant and Alkali tolerant Protein A constructs such as MabSelect SuRe (Cytiva), MabSelect PrismA (Cytiva), MaXtar® ARPA ligand Protein A (Bio-Link), recombinant Protein A (TRUKING MICRO-SPPHERE). This kit is for Research Use Only and are not intended for diagnostic use in humans or animals.

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Protein A. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Protein A, HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Protein A. You can calculate the concentration of Protein A in the samples by comparing the OD of the samples to the standard curve.

KIT COMPONENTS

Item	Specifications
ELISA Microtiter plate	96 wells
Standard substance 1	MabSelect Sube (Cytiva) (50ng/mL), 300 µL
Standard substance 2	MabSelect PrismA (Cytiva) (50ng/mL), 300 µL
Standard substance 3	MaXtar® ARPA ligand Protein A (Bio-Link) (50ng/mL) , 300 µL
Standard substance 4	Recombinant Protein A (TRUKING MICRO-SPPHERE) (50ng/mL) , 300 µL
HRP Conjugate	15 mL
Concentrated Wash Buffer (20×)	30 mL
Sample Diluent (20×)	30 mL
Substrate Reagent A	8 mL
Substrate Reagent B	8 mL
Stop Solution	15 mL
Plate Sealer	3 pieces
Sealed Bag	1
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

OTHER SUPPLIES REQUIRED

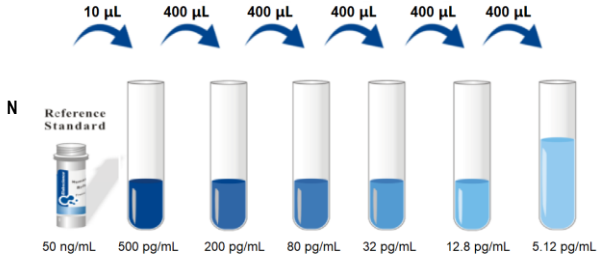
Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot

STORAGE AND EXPIRY DATE

The unopened reagent kit is stored at 2-8℃ and has a shelf life of 12 months.

REAGENT PREPARARION

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.
3. **Sample Diluent:** The **20×Sample Diluent** dilute with deionized water at 1:19.
4. Preparation of **Color Development Liquid**: **Substrate Reagent A** and **Substrate Reagent B** are mixed in equal volume.
   Note: The mixed liquid should be used within 10 min, if the color solution has turned blue after mixing, please do not use.
5. Preparation of **standard sample:**
   Please select the corresponding standard according to the type of Protein A affinity resin used to establish a standard curve.
   If the corresponding Protein A ligand cannot be obtained, for recombinant resins, the recombinant Protein A (TRUKING MICRO-SPPHERE) in this kit can be used to establish a standard curve; For alkali tolerant resins, the MabSelect SuRe (Cytiva) standard in this kit can be used to establish a standard curve.
   Diluent procedure:
   Standard substance (50 ng/mL) diluted to 500pg/mL with sample diluent firstly (1:99). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 500 pg/mL, 200 pg/mL, 80 pg/mL, 32 pg/mL, 12.8 pg/mL, 5.12 pg/mL. Dilution method: Take 5 EP tubes, add 600 µL of Sample Diluent to each tube. Pipette 400 µL of the 500 pg/mL working solution to the first tube and mix up to produce a 200 pg/mL working solution. Pipette 400 µL of the solution from the former tube into the latter one according to this step. The illustration on the next page is for reference.
   Note: A newly prepared standard solution is required for each experiment.



6. Preparation of samples:
   Sample ready to use:
   Return the sample to room temperature and mix well. Dilute the sample with sample diluent (1×). For different samples, it is necessary to verify the dilution ratio of sample concentration. The recommended sample dilution concentration range is 0.01~1mg/mL.
   Spiked sample:
   1) The samples to be tested with appropriate concentration were selected and divided into 3~4 parts of the same volume, among which 2-3 samples were added with same volume of the standard samples of different concentrations to prepare the samples to be recovered for analysis. The added volume of the standard sample should less than or equal to 10% of the total volume.
   2) The same volume of sample diluent (1×) is added to another sample to make the base sample.
   Note: It is recommended to prepare an additional 50-100 µL of sample and spiked sample.

ASSAY PROCEDURE

1. Determine wells for standard substance and sample. Add 100 µL each standard substance, and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. Recommended adding sequence: standard holes, blank holes, sample holes, spiked sample holes, standard samples are added according to the concentration gradient). Cover the plate with the sealer provided in the kit. Shock incubate (500rpm) for 60 min at room temperature.
   Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Wash: After incubation, remove the plate sealer and aspirate the liquid of each well. Immediately add 250 µL of Wash Buffer to each well and wash. Repeat the washing procedure for 3 times with wash buffer.
   Note: If using manual cleaning, immerse for 60 sec each time. Automatic washing should gently shake for 5 seconds.
3. Add 100 µL of HRP conjugate to each well. Cover the plate with a new sealer. Shock incubate (500rpm) for 60 min at at room temperature.
4. Repeat step 3.
5. Add 100 µL of Color Development Liquid to each well. Cover the plate with sealer. Incubate for 15 min at at room temperature. Avoid bubbles.
6. Add 100 µL of Stop Solution to each well. Avoid bubbles.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450/630 nm.
   Note: It is recommended to set the shock of 5-10s in the reading procedure of the micro-plate reader

PERFORMANCE

1. Limit of Detection: ＜ 5.12pg/mL;
2. Limit of Quantitation: 5.12pg/mL;
3. Linear range: 5.12-500pg/mL;
4. Accuracy (Recovery) : 80%-120%;
5. Accuracy (Relative Deviance) : ≤ 15%;

6. Repeatability (Intra CV) : ≤ 10%

CALCULATION OF RESULTS

1. OD value
   Calibration OD value = OD450nm - OD630nm – OD blank
2. Average the duplicate readings for each standard and samples. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

NOTES

1. Each component should be fully mixed before use to ensure the uniformity of the reagent. Standard substance should be centrifuged briefly for 5s, concentrate all the liquid on the tube wall and lid at the bottom of the tube, and immediately put all reagents back to 2-8℃ after use.
2. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
3. A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
4. Do not mix or substitute reagents with those from other lots or sources.
5. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
6. When the ELISA Microtiter plate are washed and patted dry, pay attention to prevent the slats from falling off, and the Plate Sealer cannot be reused.
7. High concentration may produce black flocculent during color development, which is a normal phenomenon, and the degree of slight does not affect the final reading result.
8. The kit is also suitable for recombinant, alkali tolerant protein A from different sources, and can directly detect protein A residue levels from different sources. To further guarantee the accuracy of the results, the standard curve can be established on its own using protein A from a specific source that is actually used in the production process.
9. The kit should not be used beyond the expiration date on the kit label.

FOR RESEARCH USE ONLY

Tel: 400-967-3365
Email: techsupport@uni-science.com
Website: www.elabscience.cn

Version: A 1.0 **Date Adopted: 2024.08.27**