distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com info@eaglebio.com



# Instructions for Use of Protein A ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

**Cat. No. HG- PA001**

## Introduction

BlueKit series Protein A ELISA Detection Kit is a specialized kit for quantitative detection of residual Protein A in biological products..

This kit uses the double-antibody sandwich method to coat the 96-well microtiter plate with capture antibody to prepare the solid phase antibody, add the standards and detection samples, and then add the horseradish peroxidase (HRP)-labeled microtiter antibody to form a sandwich conjugate of solid phase antibody-Protein A-microtiter antibody. After the reaction, wash the plate and then add the substrate for color reaction. The substrate converts from colorless to blue under the catalysis of HRP, and converts to yellow finally under the action of stop solution. Determine the absorbance values (OD values) at 450 nm and 630 nm, with 630 nm as the correction wavelength. OD values are positively correlated with Protein A content in the samples.

Assay range: 5.12-500 pg/mL
Limit of quantification: 5.12 pg/mL
Accuracy： 80% - 120%

## Specification

96 T

## Usage

It is applicable to the optimization of purification process of biological products, impurity control of intermediate process and release testing of final products.

## Kit components

| Components | Specification | Preparation |
|---|---|---|
| Coated Plate | 8 wells × 12 strips | Ready-to-use |
| Standard 1 (50ng/mL) | 300 μL × 1 tube | It's applicable to MabSelect SuRe ligands |
| Standard 2 (50ng/mL) | 300 μL × 1 tube | It's applicable to MabSelect PrismA ligands |
| Standard 3 (50ng/mL) | 300 μL × 1 tube | Recombinant Protein A (Truking Micro-sphere) |
| Standard 4 (50ng/mL) | 300 μL × 1 tube | MaXtar® ARPA ligand ProteinA(Bio-Link) |
| Enzyme-labelled Antibody | 15 mL x 1 vial | Ready-to-use |
| Dilution Buffer(20×) | 30 mL x 1 vial | Make a 20-fold dilution with ultrapure water. |
| Wash Buffer(20×) | 30 mL x 1 vial | Make a 20-fold dilution with ultrapure water. |
| TMB Substrate A | 8 mL × 1 vial | Ready-to-use |
| TMB Substrate B | 8 mL × 1 vial | Ready-to-use |
| Stop Solution | 15 mL × 1 vial | Ready-to-use |
| Sealing Film | 5 pieces | Ready-to-use |
| Instructions for Use | 1 copy | Ready-to-use |

## Storage and shelf life

Sealed kits are valid for 12 months at 2-8℃.

## Apparatus and materials to be prepared by the user:

- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized water
- Unused filter paper
- Vortex shaker

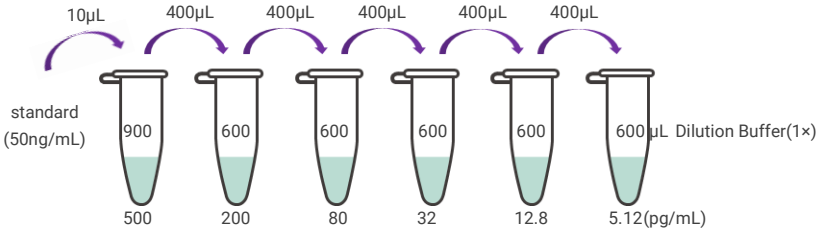
## Pre-experiment preparation

1. Preparation of 1x sample diluent: Dilute sample diluent (20×) with deionized water by a factor of 20 for later use. For example, mix 10 mL sample diluent (20×) with 190 mL deionized water.
2. Preparation of 1x washing solution: Dilute the washing solution (20×) with deionized water by a factor of 20 for later use. For example, mix 10 mL of washing solution (20×) with 190 mL of deionized water.

   Note: If crystals are formed in the sample diluent (20×) and washing solution (20×), place them at room temperature or gently shake in 37℃ water baths, and perform dilution after crystals are completely dissolved
3. Preparation of chromogenic reagents: Mix equal volumes of chromogenic reagent A and chromogenic solution B, mix well and place away from light. (Note: It cannot be left for too long and is generally prepared 10 min before use. Do not use if the mixed chromogenic reagent has turned blue).
4. Preparation of standards (Note: Freshly prepare standard solutions for each experiment)

   Different types of Protein A standards are placed in the kit. Please select the corresponding standards according to the type of Protein A affinity resin used to establish the standard curve. If the corresponding Protein A ligand cannot be obtained, the recombinant Protein A (Truking Micro-sphere) in this kit can be used to establish the standard curve for recombinant resin; The standard for MabSelect SuRe ligand in this kit can be used to establish the standard curve for the alkali-resistant resin.

   Dilution process: Dilute the standard (50 ng/mL) to 500 pg/mL with sample diluent (1×), and then dilute to prepare the standards by serial fold (dilution factor: 2.5 times), as shown below:




5. Dilution of sample to be tested: Restore the sample to room temperature and mix well; Dilute the samples with sample diluent (1×). For different samples, the dilution factor of sample concentration shall be validated. The concentration range of sample dilution is recommended to be 0.01-1 mg/mL.
6. Preparation of spiked samples: Select samples to be tested at appropriate concentrations, divide into 3-4 portions with the same volume, and add analyte standards with the same volume at different concentrations into 2-3 samples to prepare the test sample to be recovered. The added volume of analyte standard is less than or equal to 10% of the total volume, to prepare 2-3 samples to be recovered for analysis at different concentrations, and calculate the concentration of analyte standard added.

## Operation procedures

All operations are performed at room temperature, and it is recommended that all sample wells be determined in duplicate wells.

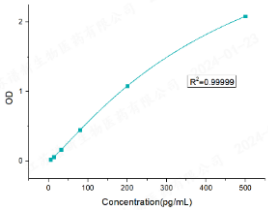
1. Restore each component of the kit to room temperature for 30 min, take out the plate strips required for the test from the aluminum foil bag that has been equilibrated to room temperature, mark the order of the plate strips with a marker, seal the remaining plate strips with a Sealing film and then put it back into the aluminum foil bag, seal and store at 2-8℃.
(Note: In the process of plate patting, the plate strips are easy to fall off, so be sure to mark.)
2. Sample incubation: Add the prepared standards and samples into the ELISA plate (recommended order of addition: standard well, blank well, sample well and spiked sample well. Add the standards according to the concentration gradient), 100 μL/well, seal the plate with the sealing film, and then incubate at 37℃ and 500 rpm for 1 h with shaking.
(Note: The samples should be added within 10 min to avoid drift with time. If the plate is not sealed or the plate is not completely sealed during incubation, it will lead to evaporation of the reaction solution, resulting in experimental errors.)
3. Plate washing: After incubation, carefully remove the sealing film, discard the liquid in the wells, wash the plate 3 times (250 μL/well) with sample diluent (1×), and pat dry the residual liquid in the wells. (If the plate is washed by hands, add sample diluent (1×), and allow to stand for 1 min; If the plate is washed using a plate washer, shake slightly for 5 s after adding sample diluent (1×).)
4. Incubation of enzyme-labeled antibody: Add enzyme-labeled antibody, 100 μL/well, seal the plate with microplate sealer, and then incubate at 37℃ and 500 rpm for 1 h with shaking.
5. Plate washing: After incubation, carefully remove the sealing film, discard the liquid in the wells, wash the plate 3 times (250 μL/well) with washing solution (1×), and pat dry the residual liquid in the wells.
6. Color development: Add the prepared chromogenic reagent into the plate by 100μL/well, seal the plate with Sealing film, and incubate at 37℃ away from light for 15 min.
7. Termination: Add stop solution, 100 μL/well, and read after color of solution is uniform (Note: It's generally completed in 10 min after adding stop solution).
8. Reading: Place the plate in the reader, set the wavelength as dual wavelength of 450/630 nm, and read the absorbance value (Note: It's recommended to set shaking for 5-10s in the reading program of microplate reader).

## Results processing

The following standard curve is only for reference, and the standard curve drawn from the standard of the same experiment should prevail:

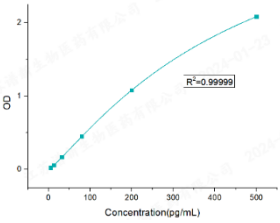
1. It's applicable to the standard curve of MabSelect SuRe ligand standard

| Standard concentration (pg/mL) | OD value |
|---|---|
| 500 | 2.0822 |
| 200 | 1.0807 |
| 80 | 0.4478 |
| 32 | 0.1671 |
| 12.8 | 0.0565 |
| 5.12 | 0.0223 |



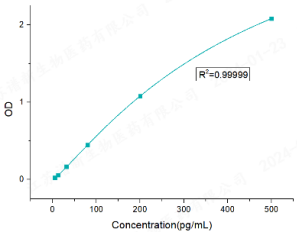

2. It's applicable to the standard curve of MabSelect PrismA ligand standard

| Standard concentration (pg/mL) | OD value |
|---|---|
| 500 | 2.6761 |
| 200 | 1.5695 |
| 80 | 0.6920 |
| 32 | 0.2735 |
| 12.8 | 0.0890 |
| 5.12 | 0.0302 |



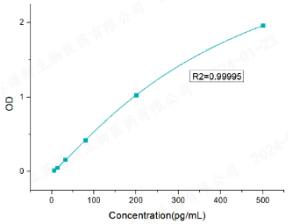
3. the standard curve of Recombinant Protein A (Truking Micro-sphere)

| Standard concentration (pg/mL) | OD value |
|---|---|
| 500 | 2.6365 |
| 200 | 1.5137 |
| 80 | 0.6550 |
| 32 | 0.2467 |
| 12.8 | 0.0858 |
| 5.12 | 0.0270 |




4. the standard curve of MaXtar® ARPA ligand Protein A (Bio-Link)

| Standard concentration (pg/mL) | OD value |
|---|---|
| 500 | 1.9603 |
| 200 | 1.0287 |
| 80 | 0.4210 |
| 32 | 0.1606 |
| 12.8 | 0.0522 |
| 5.12 | 0.0150 |




## Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
3. Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. The kit is intended for single use. Please use within the shelf life..

## Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.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Ver.1.2401
Jiangsu Hillgene Biopharma Co., Ltd.
A. Building 4, Yuewang Intelligent Valley, No. 1463, Wuzhong Avenue, Wuzhong District, Suzhou, Jiangsu, China
T. +86-18013115357 / +86-4009001882