MouSelect
Protein A binding test

Product #09-04

INTRODUCTION

Purification on Protein A is the most common method for purification of antibodies, especially in the later phases of pharmaceutical development.

The MouSelect kit contains the basic components needed to set up a sandwich ELISA for the assessment of Protein A binding. The components are designed to aid in an early selection of polyclonal or monoclonal antibodies, to a preferential purification on Protein A (from *Staphylococcus aureus*). The kit can also be used to optimise the Protein A binding of antibodies.

The information of an early screening will save valuable time down-stream, since products have already been preferentially selected towards later Protein A purification. Screening on-column or in pre-filled filter plates is more time consuming and expensive, and this procedure is generally not possible for a large number of antibodies.

KEY FEATURES

- Detection of mouse IgG1, IgG2a, IgG2b, and IgG3.
- Enough reagents included to run 8 plates, i.e. an initial screen for up to 700 samples.
- Simple operation without any sample preparation steps. Culture supernatant can generally be used without dilution.
- Possible to customize the assay to your needs.

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ASSAY PRINCIPLE

Protein A is bound to a microtiter plate. The Blocking solution, designed for low background, is added. Samples containing IgG with affinity to Protein A will bind, and can thus be detected. The Detection solution will detect mouse antibodies classes to a similar extent and will not react with Protein A.
From the screening, users can quickly select the antibodies that are most suitable for purification before further testing *in vitro* or *in vivo*.

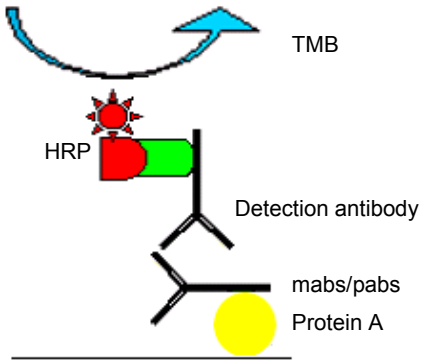

Fig 1. Assay principle.

CONTENTS

- **Coating solution (100x),** 1000 µL. Contains preservative. [YELLOW]
- **Blocking solution (100x),** 1500 µL. Contains preservative. [BLUE]
- **Positive control (100x),** 200 µL. Contains preservative. [BLACK]
- **Detection antibody (100x),** 1000 µL. Contains preservative. [GREEN]
- **HRP conjugate (100x),** 1000 µL. Contains preservative. [RED]

MATERIALS REQUIRED

- Microtiter plate
- Coating buffer, 0.1M Sodium Carbonate buffer, pH 9.5, or PBS (150 mM NaCl, 10 mM Na_2HPO_4, 2 mM NaH_2PO_4, pH 7.2).
- Washing buffer, e.g. PBS with 0.05% Tween® 20.
- TMB substrate (3,3',5,5'-tetramethylbenzidine)
- Stop solution, e.g. 1M HCl

REAGENT PREPARATION

The test has been used with the following washing buffers; PBS (pH 7.2), 0.1M Tris (pH 8.5) and 0.1M Glycine (pH 2.25). In the examples below, the amount needed to assay 1 plate is calculated. Diluted solutions should be used within 24 hours.

- **Coating solution:** Dilute 1 + 100 in coating buffer. *Example:* Mix 110 µL Coating solution (100x) with 11 mL coating buffer. Vortex.
- **Blocking solution:** Dilute 1 + 100 in coating buffer. *Example:* Mix 160 µL Blocking solution (100x) with 16 mL coating buffer. Vortex.
- **Positive control:** Dilute 1 + 100 in washing buffer. *Example:* Mix 10 µL Positive control (100x) with 1 mL washing buffer. Vortex.
- **Detection antibody and HRP conjugate:** Dilute 1 + 100 in washing buffer. *Example:* Mix 110 µL Detection antibody (100x) with 11 mL washing buffer. Vortex.

SAMPLE PREPARATION

Sample preparation is generally not needed. Depending on the amount available and the concentration of samples, dilution may be necessary and should be done in washing buffer.

Any serum that has been added to the media will bind to Protein A and can thus contribute significantly to the response. To correct for this, an identical blank media should be run along with samples.

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TEST PROCEDURE

1. Bring all reagents and samples to room temperature, 18-25˚C, before use.

2. Add 100 µL of diluted Coating solution to all wells. Cover the plate and incubate 2 hours on a shaker at room temperature.

3. Empty all wells and add 250 µL washing buffer to each well. Repeat twice for a total of three washes. After the last step, any remaining wash solution is removed by tapping the plate upside down onto a paper towel.

4. Add 150 µL of diluted Blocking solution to all wells. Cover the plate and incubate 1 hour on a shaker at room temperature

5. Wash according to step 3.

6. Add 100 µL of samples and diluted Positive control to appropriate wells. Cover the plate and incubate 1 hour at room temperature.

7. Wash according to step 3.

8. Add 100 µL of diluted Detection antibody to each well. Cover the plate and incubate 1 hour at room temperature.

9. Wash according to step 3.

10. Add 100 µL of diluted HRP conjugate to each well. Cover the plate and incubate 30 minutes at room temperature.

11. Wash according to step 3.

12. Add 100 µL of Substrate solution (TMB) to each well and incubate the plate 10 minutes at room temperature in the dark. Begin timing after the first well is filled.

13. Stop the reaction by adding 100 µL Stop solution (e.g. 1M HCl) to each well. Add the Stop solution in the same order as the Substrate solution was added.

14. Measure the absorbance of the samples and standard references at 450 nm within 30 minutes after adding the Stop solution.

ANALYSIS OF RESULTS

For a Protein A binding assessment, the output of each sample is related either to the positive control or to the other samples in the plate.

Deduct the plate blank, or the media specific blank, from each well. Correlate each sample to a percentage of the Positive control.

OD_{450} (Sample) / OD_{450} (Pos ctrl) * 100%

The Positive control should be considered a fair binder. Good binders generally display results >200%.

PERFORMANCE

An excerpt of test results is shown below. The performance information should only be taken as guidance. For quality control purposes, the assay should be validated in-house with respect to common parameters.

- **Validity of assay**: The Positive control should have a corrected absorbance value greater than 0.2.

- **Sample graph:** Shows the concentration dependent response in the assay. The curve is for demonstration purposes only.

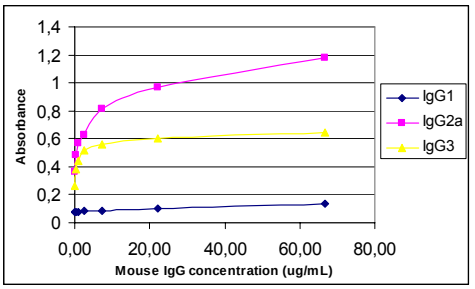

Fig 3. Detection of mouse antibodies bound to Protein A in a sandwich ELISA.

- **Binding:** Different SpA sources, both native and recombinant, have been tested. This did not have a key impact on the results. In the noted results below, we have used SpA from GE Healthcare.

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- **Specificity:** The detection antibodies will detect all four classes of mouse IgG to a similar extent. The antibodies will also cross-react with other mammalian antibodies, but variability between these classes has not been tested for.

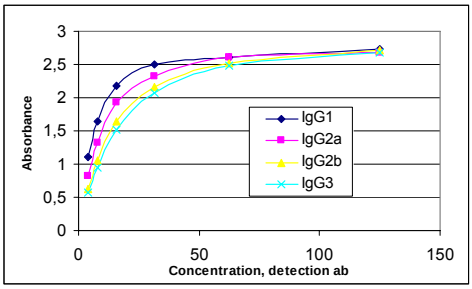

Fig 2. Detection of mouse antibody classes (bound in plate) in a direct ELISA.

- **Precision:** Coefficients of variation (CV) for duplicate measurements are typically <10%.

- **Antigen excess:** No typical hook effect was seen for the Positive control when analyzed up to 1000 µg/mL.

- **Matrix effect:** When serum is not added, commonly used media (tested with RPMI, MEM and DMEM media) do not give any additional background in the assay and samples spiked gave response similar to simple buffer dilutions.

- **Binding optimization:** The assay can be used over a wide pH-range and can therefore be used to optimize the binding buffer as well as the elution buffer.

TECHNICAL DATA

Storage: +2 to +8°C (35 to 45°F).

Shelf life: For unopened kit, see expiry date on package. Within this time, opened vials can be used for up to 6 months if refrigerated between each usage.

HINTS AND LIMITATIONS

- **Quantification:** To determine the exact binding capacity of your samples, a standard curve should be prepared for each antibody of interest and analyzed alongside the samples in the plate. Use data reduction software to generate an appropriate standard curve.

PRECAUTIONS

Product MSDS can be found on the homepage as indicated below.

FOR RESEARCH USE ONLY.

ORDER INFORMATION

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