STANDARD OPERATIONS PROCEDURES FOR THE COMMON FUND: PROTEIN CAPTURE REAGENTS PROGRAM (ELISA)

1. PURPOSE

This procedure is to be used for the characterization of purified monoclonal antibody.

2. SCOPE

This document describes the procedure for a **typical** indirect Enzyme - Linked Immuno-Sorbent Assay (ELISA) that is used to assess the reactivity of human fragment antigen-binding (Fab) or monoclonal antibodies for the recombinant antigens used as immunogens. It is not intended as a procedure for measurement of native antigens. It is also not to be used as the procedure for assessment of reactivity in unpurified monoclonal antibodies.

3. RESPONSIBILITIES

It is the responsibility of the person(s) performing this test to be familiar with lab safety procedures and to have basic laboratory skills. The interpretation of the results must be done by a person trained in the procedure and familiar with such interpretation.

4. EQUIPMENT

- Plate Shaker; Boekel Scientific Jitterbug model 130000
- Plate Washer; Bio-Rad Immunowash model 1575
- Plate Reader; Bio-Rad iMark microplate reader (450nm filter)

5. MATERIALS

- Microtiter plates – medium binding; Costar, Cat. #9017
- Carbonate Coating Buffer (pre-mixed powder) – prepared according to manufacturer's directions; Pierce-Thermo, Cat. #28382
- Phosphate Buffered Saline (PBS), 10x solution (Fisher Scientific, Cat. #BP399-1) diluted to 1x with deionized water to give 11.9 mM phosphate, 137mM NaCl, 2.7mM KCl and pH 7.4

- PBS – 0.05% Tween (PBST) prepared by addition of 500 µL Tween 20; (ACROS ORGANICS, Cat. #233362500) into 1 liter of PBS
- Blocker™ Casein in PBS, 1L; Thermo Scientific, Cat. # 37528
- 1-Step TMB Turbo Substrate; Pierce-Thermo, Cat. # 34022
- 0.18N Sulfuric acid; Thermo Scientific, Cat. # N600.
- Polypropylene Tubes

6. REAGENTS

6.1. For mouse monoclonal antibody evaluation

- Antigen corresponding to the antibody.
- Purified monoclonal antibody corresponding to the Antigen
- Goat anti-mouse - horseradish peroxidase (GAM-HRP) labeled antibody; Jackson ImmunoResearch Laboratories, Cat. # 115-035-166

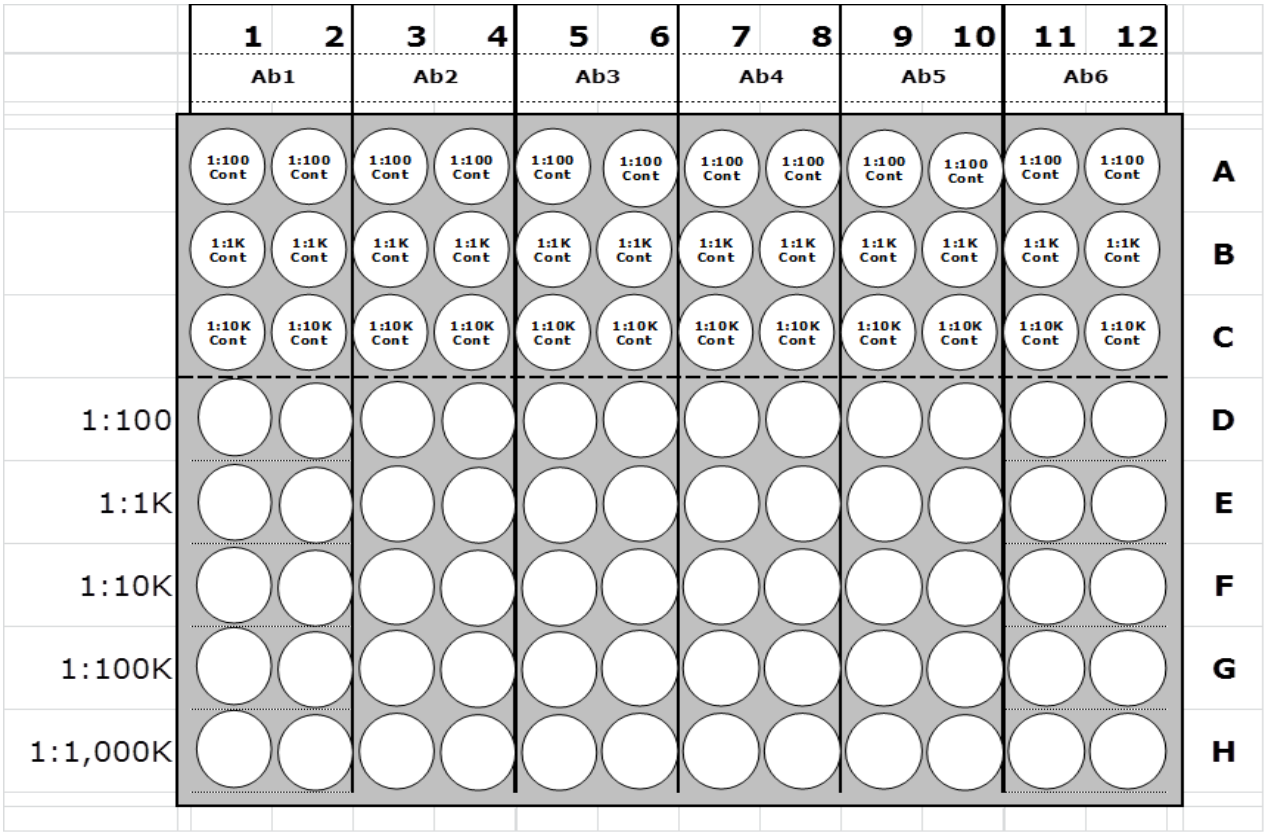
6.2. For human Fab binder evaluation

- Antigen corresponding to the antibody.
- Purified human Fab antibody corresponding to the Antigen
- Goat anti-human - horseradish peroxidase (GAH-HRP) labeled antibody; Jackson ImmunoResearch Laboratories, Cat. #109-036-097

7. PROCEDURE

7.1. Antigen Coating

7.1.1. Prepare a plate outline as in the diagram below. This format allows for up to 6 antibodies to be tested with one antigen and each antibody dilution is tested in duplicate. Control wells are located in rows A-C.



7.1.2. Prepare a dilution of the antigen into the Carbonate Coating Buffer to give a final concentration of approximately 10 µg/ml (need 100 µl/number wells).

7.1.3. Mix thoroughly by vortex.

7.1.4. Add 100 µl of diluted antigen (10 µg/ml stock) to each titration well (total 10 wells per antibody). These are indicated in the above diagram (Rows D-H).

7.1.5. Add 100 µl of Carbonate Coating Buffer to each of the control wells as indicated in the above diagram (Rows A-C).

7.1.6. Incubate the plate for 1 hour at 37°C with mild shaking using a Jitterbug shaker (320 rpm). Alternatively, incubate 16-24 hours at 4 -8°C without shaking.

7.1.7. Remove the Carbonate Coating Solution by decanting and tapping gently on a paper towel.

7.1.8. Immediately, add 225 µl of Blocker™ Casein in PBS to all wells as a blocking agent to prevent non-specific binding.

7.1.9. Incubate for 30 minutes at 37°C with mild shaking using a Jitterbug shaker (320 rpm).

7.1.10. Remove the Blocker™ Casein in PBS by decanting and tapping gently on a paper towel.

7.1.11. Rinse and wash the wells 3 times with 250 µl PBST. To do this, dispense PBST into wells and then gently decant/tap the plate on a paper towel. Repeat 3x. Alternatively rinse and wash the plate using a Plate washer.

7.2. Antibody preparation and incubation

7.2.1. Prepare 1ml of intermediate stock solution (IS) to approximately 0.01 mg/ml in PBS. Do NOT use polystyrene tubes. Polypropylene tubes are preferred for all antibody dilution steps.

Note: IS preparations may need to be adjusted depending on supernatant and antibody stock concentrations.

7.2.2. Prepare 5 dilution levels of each IS antibody in PBS as follows:

- 1:100 – IS
- 1:1K – 100 µL of IS + 900 µL of PBS
- 1:10K – 100 µL of 1:1K + 900 µL of PBS
- 1:100K – 100 µL of 1:10K + 900 µL of PBS
- 1:1000K – 100 µL of 1:100K + 900 µL of PBS

Note: Dilution levels may need to be adjusted depending on supernatant and antibody IS concentrations.

7.2.3. Add 100 µl of each dilution as indicated in the diagram above (1:100 to rows A and D, 1:1K to Rows B and E, 1:10K to Rows C and F, 1:100K to Row G and 1:1,000K to Row H).

7.2.4. Incubate for 30 minutes at 37°C with shaking.

7.2.5. Wash the plate: decant and rinse 3 times with PBST as described in 7.1.11.

7.3. Secondary antibody

7.3.1. Prepare a 1:7500 dilution of the GAM-HRP or GAH-HRP antibody.

7.3.2. Add 4 µl of the GAM-HRP or GAH-HRP antibody to 30 ml of PBST. This will make sufficient secondary antibody for 3 microtiter plates.

Note: Concentration of the secondary antibody may need to be adjusted depending on the individual antibody performance.

7.3.3. Add 100 µl of secondary antibody to each well as indicated in the diagram (Rows A-H, columns 1-12).

7.3.4. Incubate the plate for 30 minutes @ 37°C with mild shaking.

7.3.5. Remove the secondary antibody solution by decanting and tapping gently on a paper towel.

7.3.6. Wash the plate: decant and rinse 3 times with PBST as described in 7.1.11.

7.4. Detection and Evaluation

7.4.1. Add 100 µl of TMB substrate to each well of the plate.

7.4.2. Incubate at room temperature until sufficient blue color develops in the test wells. This time is usually 5 minutes but an additional 2 minutes may be required for weak antibodies.

7.4.3. Add 100 µl of 0.18N sulfuric acid to each well to stop the reaction. The color will change to yellow.

7.4.4. Read the absorbance in a microplate reader using a 450 nm filter.

7.4.5. Perform data analysis.

8. REFERENCED DOCUMENTS

8.1.1. Operation manual, Bio-Rad iMark microplate reader

8.1.2. Operation manual, Bio-Rad Immunowash microplate washer

8.1.3. Operation manual, Boekel Scientific Jitterbug shaker/ incubator

8.1.4. Instructions for use, Pierce 1-Step TMB Turbo Substrate