STANDARD OPERATING PROCEDURE

Title: Evaluation using Indirect ELISA

SOP#: M-102

Version #: 1 **Author: R. Saul / G. Whiteley**

Date Approved: Feb. 6, 2009 **Date Modified:**

1. PURPOSE

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

2. SCOPE

This document describes the procedure for the indirect Enzyme Linked ImmunoSorbant Assay (ELISA) that is used to assess the reactivity of the 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; BioRad Immunowash model 1575
- Plate Reader: BioRad 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, pH 7.4
- PBS – 0.05% Tween (PBST) prepared by addition of 500uL Tween 20; (ACROS Cat. #23336-2500) into 1 liter of PBS
- PBS – 0.05% Tween, 0.1% BSA (PBST-BSA) prepared by addition of 1 gram of BSA (Sigma Cat. # A7030) into 1 liter of PBST
- 1-Step TMB Turbo Substrate, Pierce-Thermo catalog # 34022
- 0.18N Sulfuric acid; Thermo Scientific, cat.# N600.
- Polypropylene Tubes

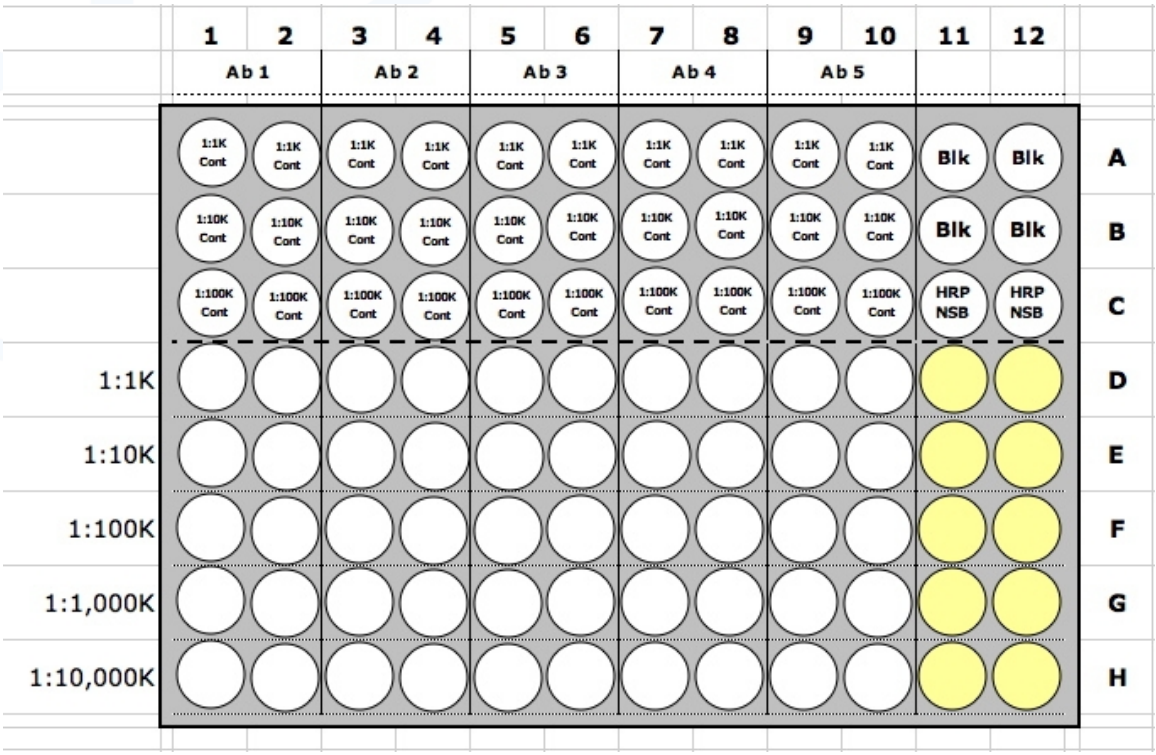
6. REAGENTS

- 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

7. PROCEDURE

7.1. Antigen Coating

7.1.1. Prepare a plate outline as in the diagram below. This format allows for up to 5 antibodies to be tested with one antigen and each antibody dilution is tested in duplicate. Control wells are located in the last two columns, rows A-C.



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

7.1.3. Mix thoroughly by vortex.

7.1.4. Add 100µl of diluted antigen (10µg/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 100µl 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. 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 225µl of PBST-BSA 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

7.1.10. Remove the PBST-BSA by decanting and tapping gently on a paper towel.

7.1.11. Rinse and wash the wells 3 times with 250µl 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.

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

- 1:1K – 100µL of IS + 900µL of PBS
- 1:10K – 100µL of 1:10 + 900µL of PBS
- 1:100K – 100µL of 1:100 + 900µL of PBS
- 1:1,000K – 100µL of 1:1000 + 900µL of PBS
- 1:10,000K – 100µL of 1:10000 + 900µL of PBS

7.2.3. Add 100µl of each dilution as indicated in the diagram above (1:10, to rows A and D, 1:100 to Rows B and E, 1:1000 to Rows C and F, 1:10000 to Row G and 1:100000 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 antibody.

7.3.1.1. Add 4µl of the GAM-HRP antibody to 30ml of PBST. This will make sufficient secondary antibody for 3 microtiter plates. Note, the concentration of the secondary antibody may need to be adjusted depending on the individual antibody performance.

7.3.2. Add 100µl of secondary antibody to each well as indicated in the diagram (Rows A-H, columns 1-10). Additionally, include the 2 additional wells listed as “HRP NSB” in the plate diagram (Row C, columns 11 and 12).

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

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

7.3.5. 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 100µl of TMB substrate to each well of the plate including the wells in columns 11 and 12 (controls).

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 100µl 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 reduction according to SOP D-100.

8. REFERENCED DOCUMENTS

8.1.1. Operation manual, Biorad iMark microplate reader

8.1.2. Operation manual, Biorad 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

8.1.5. SOP D-100: Data reduction of Indirect ELSIA results