

**SOP: AB106.1**  
**Modified: 7/14/22 KE**

### **Isotyping of Hybridoma Monoclonal Antibodies**

#### **Materials and Reagents:**

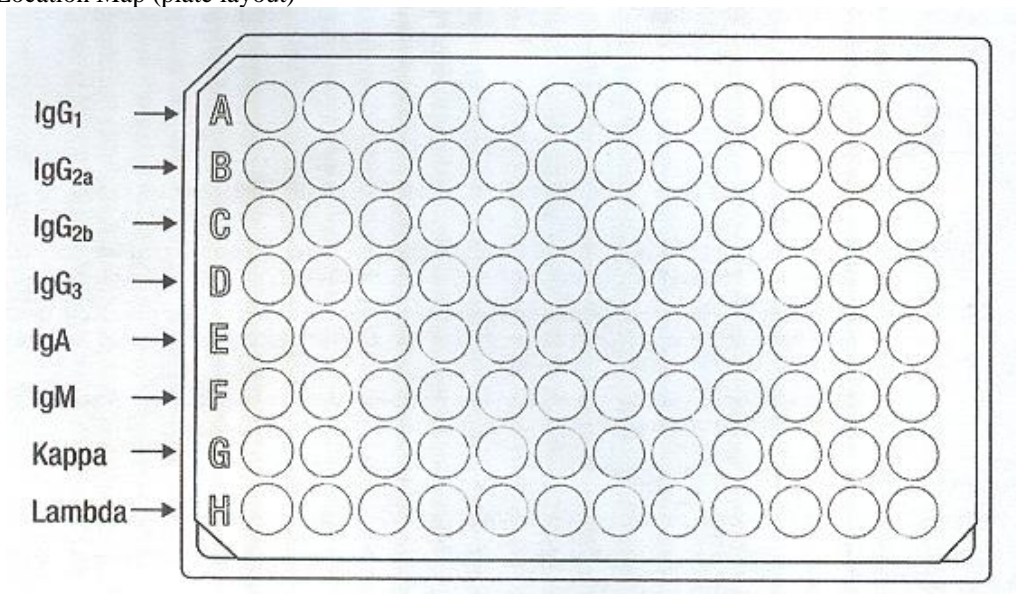
1. Pierce Rapid ELISA Mouse mAb Isotyping kit (ThermoFisher. Invitrogen Cat# 37503)
  - *Protocol based off kit manual (see references for link)*
2. TBS
3. Multichannel pipet with 200  $\mu$ L pipet tips (*use filter tips if handling biological/biohazardous fluids*)
4. Reagent reservoirs
5. 0.65 mL Eppendorf tubes
6. Plate sealers (clear, sterile OR non-sterile, NOT PCR sealers)
7. Titer plate shaker (Lab-Line Instruments, Inc., Model #4625)
  - *ALL shaking steps should be performed at 200 rpm (speed "2" on titer plate shaker)*
8. Microplate reader
  - *Plate reader should be able to read absorbance*

#### **Protocol:**

- 1.\_\_\_\_\_ Obtain a small aliquot of hybridoma *supernatant* or purified antibody.
- 2.\_\_\_\_\_ Prepare the antibody to be tested by diluting in TBS as follows:
  - Hybridoma supernatants: dilute sample 1:50 in TBS: add 20  $\mu$ L of hybridoma supernatant to 980  $\mu$ L of TBS. The suggested assay dilution range is 1:10-1:100.
  - Purified antibody: dilute sample to 250 ng/mL in TBS. The suggested assay concentration range is 25 ng/mL-2  $\mu$ g/mL
- 3.\_\_\_\_\_ Equilibrate TMB substrate and plate strips (from kit) to room temperature.
- 4.\_\_\_\_\_ Add 50  $\mu$ L of diluted antibody sample to each well of the 8-well strip.
- 5.\_\_\_\_\_ Add 50  $\mu$ L of the Goat Anti-Mouse IgG+IgA+IgM HRP Conjugate (from kit) to each well of the 8-well strip. Mix by gently tapping the plate or by gentle mixing on a plate shaker.
- 6.\_\_\_\_\_ Cover plate and incubate at room temperature for 1 hr.
- 7.\_\_\_\_\_ Decant plate contents (Note 1).
- 8.\_\_\_\_\_ Wash plate thrice with 200  $\mu$ L/well of wash buffer (from kit) (Note 2).
- 9.\_\_\_\_\_ Add 75  $\mu$ L of TMB Substrate (from kit) to each well. A blue positive response may be visible after 1 min. Signal development and intensity varies depending on antibody concentration and isotype.
- 10.\_\_\_\_\_ After 5-15 minutes add 75  $\mu$ L of Stop Solution (from kit). The Stop Solution changes the color from blue to yellow.
- 11.\_\_\_\_\_ Read at 450 nm on a microplate reader.
- 12.\_\_\_\_\_ Interpret results using the location map below (Note 3). Each sample should have a positive response in **one of the rows A-F (heavy-chain identification)** and a positive response in **either row G or H (light-chain identification)** (Note 4).

**Notes:**

1. ***If plate contents contain biological/biohazardous waste, the liquid must be handled and disposed of properly as biohazardous waste (cannot be decanted into sink).*** However, if plate contents do NOT contain any biological/biohazardous waste, then the liquid can be decanted directly into sink. Make sure to check wells are void of residual liquid (can tap microplate a couple more times). Make sure to tap on *dry spot* of towel after each wash as to not contaminate microplate with backsplash of liquid on towel.
2. Washes should be performed ***quickly***. Wash buffer should be added using a multichannel pipet (with 200  $\mu$ L capacity), gently tapped, then decanted into sink (3-4 shakes). ***IF washes are performed after an incubation with biological fluids/biohazardous waste, the washes must be handled and disposed of properly as biohazardous waste (cannot be decanted into sink).*** The microplate should be tapped 3-4 times on a towel for liquid removal. Make sure to check wells are void of residual liquid (can tap microplate a couple more times). Make sure to tap on *dry spot* of towel after each wash as to not contaminate microplate with backsplash of liquid on towel.
3. Location Map (plate layout)



4. Wells with the highest response (darkest color) indicate isotype and light chain composition; lightly colored wells indicate contaminating host or myeloma antibodies.

**References:**

1. Pierce Rapid ELISA Mouse mAb Isotyping Kit Manual: [https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FMSG%2Fmanuals%2FMAN0014495\\_37503\\_Ms\\_mAb\\_Isotyping\\_Pierce\\_RapidELISA\\_UG.pdf](https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FMSG%2Fmanuals%2FMAN0014495_37503_Ms_mAb_Isotyping_Pierce_RapidELISA_UG.pdf)