Background

Early characterization of a monoclonal antibody's isotype is important for a number of reasons. First, an antibody's isotype can determine the simplest method of purification. For example, IgG_{2a} and IgG_{2b} bind Protein A and, thus can be purified by Protein A affinity chromatography; however, this method may not be suitable for IgG_1 monoclonals because they do not bind Protein A well under standard conditions. Second, some isotypes are better suited to certain immunological techniques than others. For example, IgG_{2b} antibodies most effectively stimulate complement, making them the best isotype for *in vivo* immunotherapy studies and for complement-mediated killing of *in vitro* cells carrying the epitope. Similarly, purification and enzyme conjugation of IgG_3 and IgM antibodies can be difficult, making single-step applications unlikely. Third, isotype characterization also reveals an antibody's structure, which may make it undesirable in some applications. For example, IgM's exist as pentamers composed of five 180-kD subunits; therefore, these unstable IgM monoclonals are often too large for applications that require monomers, such as the IgG isotypes or the majority of IgA's. Fourth, an antibody's isotype determines the best method of preparing Fab fragments by proteolysis (*e.g.*, using papain digests for IgG's).

Kit Principle

Isotype characterization with the IsoStrip Kit is made simple by the kit's two major components. Each development tube supplied with the kit contains latex beads bearing anti-mouse kappa and anti-mouse lambda antibodies, which will react with any mouse monoclonal antibody regardless of its isotype. The basis of the kit is the isotyping strip itself, which bears immobilized bands of goat anti-mouse antibodies corresponding to each of the common mouse antibody isotypes (IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgM, and IgA) and to the kappa and lambda light chains. Both sides of the strip also bear a positive-control band, which indicates that the antibody-coated beads have traveled up the strip.

Using these two components, a mouse monoclonal antibody can be screened for isotype by simply diluting the antibody sample, pipetting the diluted sample into the development tube, and inserting the strip. Diluting the ascites fluid or tissue culture supernatant reduces the concentration of both contaminating host immunoglobulins and the desired monoclonal antibody of unknown isotype; when the starting concentration of the desired antibody is greater than those of the contaminants, the antibody of interest will appear as a clear, dominant band on this sensitive strip, but bands caused by the contaminants will be faint or not appear at all. This diluted sample is then added to the development tube, where the mouse monoclonal antibody resuspends and forms a complex with the antibody-coated latex beads. When the isotyping strip is placed in the development tube, this complex flows up the strip (via capillary action) until it is bound by the immobilized goat anti-mouse antibody specific for the monoclonal's isotype and by the immobilized antibody specific for the monoclonal's light chain.

In approximately 5–10 minutes, latex beads aggregate as blue bands in the two sections corresponding to the monoclonal antibody's isotype and to the nature of its light chain. Development of the strip is complete when the positive-control band on each side of the strip turns blue.

Changes to previous version Editorial Changes.

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IsoStrip

Mouse Monoclonal Antibody Isotyping Kit

Cat. No. 11 493 027 001 1 kit for 10 tests

Version 06
Content version: March 2018
Store at +2 to +8°C

Product Description

Roche IsoStrip Mouse Monoclonal Antibody Isotyping Kit is a simple, three-step kit for the rapid characterization of mouse monoclonal antibodies.

Kit contents
- 10 capped development tubes, each containing lyophilized latex beads
- 10 isotyping strips in a canister

Storage and stability The kit contents are stable through the control date printed on the kit label when stored at +2 to +8°C.

Procedure

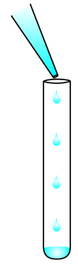
1. Dilute Sample
- Remove the desired number of isotyping strips from the canister. Remove the caps from an equal number of development tubes.

  The tubes may be labeled with a pencil or felt-tipped lab marker for easy identification.
- Dilute a sample containing the mouse monoclonal antibody in 1% BSA/ phosphate-buffered saline (PBS), pH 7.2–7.6.

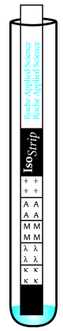
  Culture supernatant samples should be diluted 1:10 to 1:100. Ascites samples should be diluted 1:20,000. These are recommended dilutions and may vary depending on the concentration of antibody in your sample. In our experience, a monoclonal antibody concentration of 0.1–1 µg/ml of diluted sample gives the best results.

  150 µl of this diluted sample will be added to the development tube.

2. Pipette sample into tube
- Pipette 150 µl of the **freshly diluted** sample into each development tube. Incubate at +15 to +25°C for 30 seconds, then briefly agitate (*e.g.*, vortex) the tube so that the colored latex is completely resuspended.

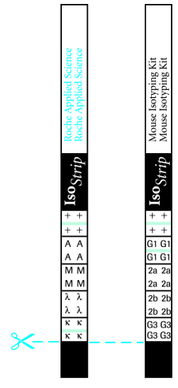


3. Insert strip in development tube
- Place one isotyping strip, with the black end at the bottom, in each development tube for at least 1 minute.



Interpreting results

Interpret the results after 5–10 minutes (the whole strip should be wetted with the sample and the positive control bands have appeared). Do not wash the strip to stop the reaction.



Within 5–10 minutes, a blue band will appear in either the kappa or lambda section of the strip, as well as in one of the class or subclass sections, indicating the class or subclass and light-chain composition of the monoclonal antibody. These blue bands will intensify as the sample moves up the strip. The positive control bands on each side of the isotyping strip should also appear, indicating that the antibody-coated latex beads are functional and have traveled up the strip.

In some cases (*e.g.*, when testing very dilute samples), the test may take up to 10 minutes.

Note: For a permanent experimental record or for an easier interpretation of results when testing samples containing host immunoglobulins (see pages 2 and 3 for "Further Information on Interpretation of Results"), the black area may be cut off of the bottom of the strip to prevent further band development once the positive control bands are visible.

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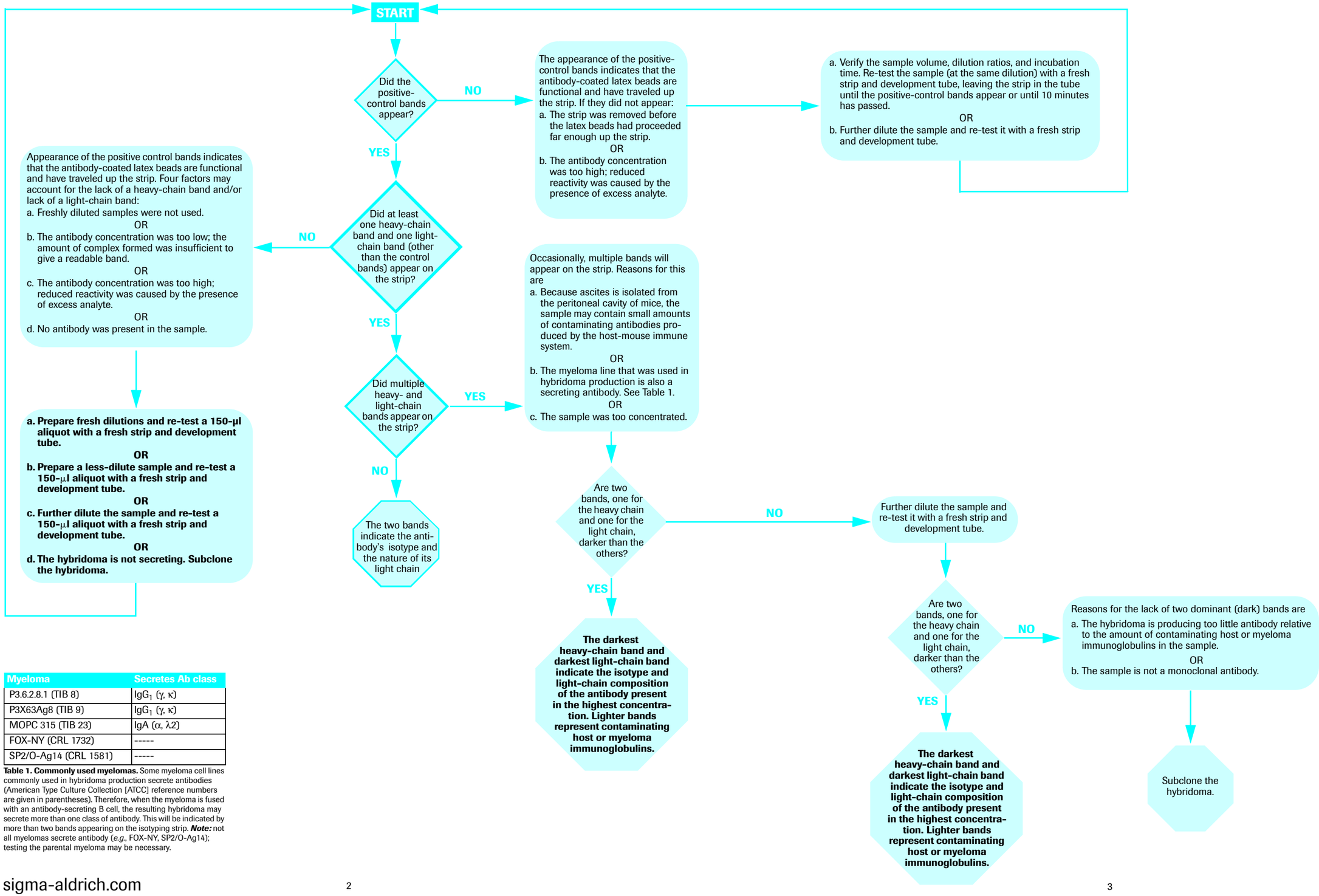
Further Information on Interpretation of Results

Typically, four blue bands will appear, one indicating the antibody's class or subclass, one reflecting the nature of its light chain, and two corresponding to the positive controls. However, with some samples, different numbers of bands may appear on the strip. In these cases, use the flowchart below to interpret your results.



Myeloma	Secretes Ab class
P3.6.2.8.1 (TIB 8)	IgG_1 (γ, κ)
P3X63Ag8 (TIB 9)	IgG_1 (γ, κ)
MOPC 315 (TIB 23)	IgA (α, λ2)
FOX-NY (CRL 1732)	-----
SP2/O-Ag14 (CRL 1581)	-----

Table 1. Commonly used myelomas. Some myeloma cell lines commonly used in hybridoma production secrete antibodies (American Type Culture Collection [ATCC] reference numbers are given in parentheses). Therefore, when the myeloma is fused with an antibody-secreting B cell, the resulting hybridoma may secrete more than one class of antibody. This will be indicated by more than two bands appearing on the isotyping strip. ***Note:*** not all myelomas secrete antibody (*e.g.*, FOX-NY, SP2/O-Ag14); testing the parental myeloma may be necessary.