KAMIYA BIOMEDICAL COMPANY

Mouse Isotyping Kit

For rapid identification of mouse immunoglobulin isotypes, subtypes and light chains

Cat. No. KT-789

For Research Use Only.

PRODUCT INFORMATION

Mouse Isotyping Kit
Cat. No. KT-789

INTENDED USE
The one-step immunochromatographic mouse isotyping kit allows for quick and easy identification of mouse isotypes, subtypes and light chains from a single sample. The sample used can be purified antibody, cell culture supernatant or ascites fluid. **This product is for research only, not for use in diagnostic applications.**

ADDITIONAL USE FOR THE KIT
For purified antibody and cell culture supernatant the test can also be used to determine the relative purity of a hybridoma cell line.

PRINCIPLE
Lateral-flow membranes are impregnated with separate lines containing antibodies specific for unique amino acid sequences contained within IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, kappa light chains and lambda light chains. When the isotyping strips are added to a test tube containing a diluted mouse immunoglobulin sample, colloidal gold nanoparticles conjugated with antibody form a soluble immune-complex. The complex is apparent in the form of a red band which allows users to make a qualitative determination for their specific sample.

COMPONENTS
Each kit is suitable for running 20 unique samples.

1. A single canister containing 20 **red strips** to identify - IgG1, IgG2a, IgG2b and IgG3 subtypes.
2. A single canister containing 20 **blue strips** to identify - kappa light chain, lambda light chain, IgA and IgM.
3. A single bottle containing 15 mL of sample buffer.
4. Each canister contains a single desiccant pouch and they should not be discarded.

STORAGE
Store all components at room temperature (18° to 25°C).
Do not freeze any kit components.
Kit components should not be exposed to UV light or prolonged heat.
Store all components in their original packaging.
Stable for one (1) year from the date of shipment.

PROCEDURE
For Cell Culture Fluids:

1. Bring all reagents to room temperature.
2. Remove the required number of strips from each canister. **Close canister securely.**
3. Add 375 microliters of sample buffer to each 12 x 75 mm polystyrene or glass test tube.
4. Add 25 microliters of your sample of cell culture fluid to the test tube containing 375 microliters of sample buffer and vortex to mix. This is a 1/16 dilution of your sample.
5. Add to the test tube one red strip (G1, IgG2a, IgG2b, and IgG3) and one blue strip (kappa, lambda, IgA and IgM). The blank side of each strip should be touching the other so both outward facing strips can be read while positioned in the test tube.
6. Allow the strips to incubate in the test tubes for up to 10 minutes prior to reading.

For Purified Antibodies above 1.0 mg/mL or Ascites Fluid:

1. Bring all reagents to room temperature.

2. Remove the required number of strips from each canister. **Close canister securely.**

3. Pipet 45 microliters of sample buffer to the first 12 x 75 mm polystyrene or glass test tube.

4. Add 5 microliters of your purified antibody or ascites sample to the first test tube containing 45 microliters of sample buffer and gently mix. This is a 1/10 dilution of your sample.

5. Into a second test tube pipet 395 microliters of sample buffer.

6. Add 5 microliters of the previously mentioned 1/10 sample dilution to 395 microliters of sample buffer in second test tube. This is a 1/800 dilution of your sample.

7. Add to the second test tube one red strip (G1, IgG2a, IgG2b and IgG3) and one blue strip (kappa, lambda, IgA and IgM). The blank side of each strip should be touching the other so both outward facing strips can be read while positioned in the test tube.

8. Allow the strips to incubate in the test tubes for up to 10 minutes prior to reading.

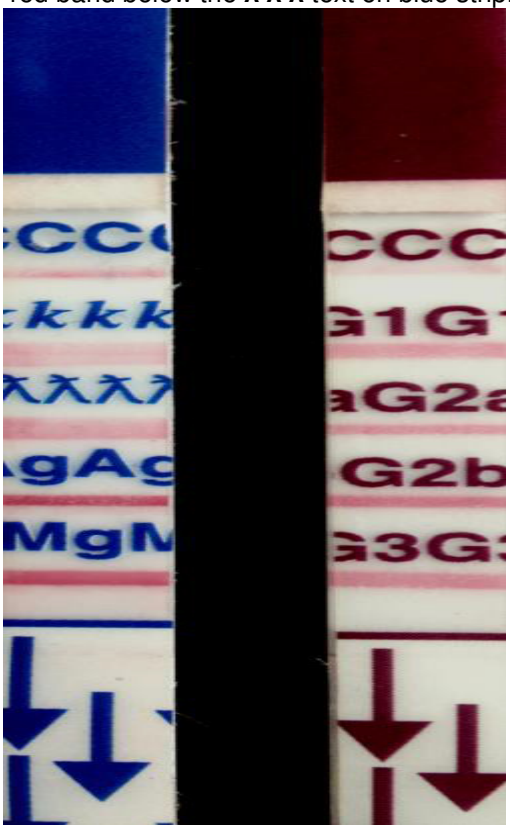
INTERPRETATION OF RESULTS

1. Interpret the results at 8-10 minutes once the positive control band appears.

2. The test is valid if a positive control band appears below the "CCC" region of the strip (see image below).

3. A colored red band will form **below** the printed text indicating the isotype, subtype and light chain of the monoclonal antibody.

4. a) Positive for IgG1 = red band below the **G1** text on red strip.
   b) Positive for IgG2a = red band below the **G2a** text on red strip.
   c) Positive for IgG2b = red band below the **G2b** text on red strip.
   d) Positive for IgG3 = red band below the **G3** text on red strip.
   e) Positive for IgA = red band below the **Ag** text on blue strip.
   f) Positive for IgM = red band below the **Mg** text on blue strip.
   g) Positive for Kappa Light Chain = red band below the **κ κ κ** text on blue strip.
   h) Positive for Lambda Light Chain = red band below the **λ λ λ** text on blue strip.



TROUBLESHOOTING

Q: Multiple bands appear on a single strip.
A: Antibody concentration is too high. Dilute sample and test again.
A: May be an indication of a mixed clone in your cell culture supernatant. Re-cloning the hybridoma may be necessary.

Q: No heavy and/or light-chain appears on either strip, but a positive control band is visible.
A: Antibody concentration of sample is too low or hybridoma isn't secreting immunoglobulin. Re-test the sample at a higher concentration.

Q: Positive result for Kappa or Lambda, but negative for IgG subtype, IgA or IgM.
A: Secreted antibody may be IgE, IgD or IgG2c.

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