

Disp&FLOW® – Rapid Isotyping for Mouse Ig

Cat. No.: BIO.065.25 **Number of tests: 25**



Intended Purpose

The Disp&FLOW® Rapid Isotyping test for mouse antibodies (immunoglobulins or Ig) is a rapid immunochromatographic, lateral-flow test designed for a quick and easy qualitative identification of mouse Ig heavy chain classes and subclasses, and light chain types from a limited volume sample. The sample used can be purified antibody or cell culture supernatant (or ascites fluid; note however that this fluid is usually a complex mixture that may include diverse array of cells and proteins, including contaminating immunoglobulins, cytokines and growth factors). For purified antibody and cell culture supernatant the test can also be used to determine the relative purity of a hybridoma cell line. This product is `For Research Use Only. Not for use in diagnostic procedures.' (RUO).

Antibodies, also known as immunoglobulins (Ig), can be categorized according to several backbones called isotypes or classes, each with unique properties and physiological functions. Isotypes refer to the variations or genetic and proteomic differences in the heavy chain constant region and determine the different classes and subclasses of Ig. In rodents (as in most animal species including humans), each individual expresses the complete collection of isotypes, but in various proportions and at different moments in its life. Altogether, IgG is the most abundant antibody isotype in serum and, in mice, it is a mix of five subclasses: IgG1, IgG2a, IgG2b, IgG2c, and IgG3. IgM is also frequent as it is often the first antibody class produced during the primary immune response, before a swich of expression occurs towards another class, like the IgGs. Next, the IgA class is mainly found in mucosal secretions. In addition to the heavy chain classes, the light chains are segregated into two types, namely kappa (κ) and lambda (λ). In \emph{vivo} , antibodies with different isotypes and light chain types differ in their biological properties and ability to engage different immunological functions. When monoclonal antibodies are used *in vitro* as analytical tools, the different isotypes and classes result in different physicochemical attributes. Thus, screening for the isotope and light chain types is of capital interest during the production of Ig.

Mode Of Action

The Disp&FLOW® Rapid Isotyping test is an immunological lateral flow screening assay that **characterizes an antibody in 10 minutes**. The assay device is in a dipstick format. In the assay, the diluted sample is allowed to migrate on a series of capillary beds. Following its application to the sample pad, the sample is discharged onto the pre-treated conjugate pad. Mouse antibody isotype conjugate is released upon contact with the flowing sample and forms soluble complexes with the analytes. These complexes travel the length of the membrane and are captured in the test region by anti-isotype antibody immobilized on the nitrocellulose membrane. A line formed in the test region indicates the presence and class, subclass, or light chain type of mouse antibodies in the test specimen. Absence of the line in the test region indicates a negative result. A line in the control region will always appear and indicates a properly functioning test.

Storage And Stability:

- Store the kit box at +2°C to +30°C in a dry environment.
- Do not freeze or expose to elevated temperatures.
- Keep all test strips sealed in the desiccated tube when not in use.
- When stored as detailed above, the Disp&FLOW® Rapid Isotyping kit and components are stable up to the expiration dates printed on the labels.
- · Discard any remaining components after their expiration.

Precautions And Warnings:

- Read all instructions before use.
- Each test strip is designed for a single use.
- Treat all specimens and any material coming into contact with them as potentially infectious.
- Wear disposable gloves when handling specimens and kit components.
- Do not mix components of one kit lot with components from other lots. Do not use kit components beyond their expiration dates.
- Do not interpret results more than 20 minutes after the start of migration.
- Do not use reagents that show signs of contamination.
- Good Laboratory Practices should be employed to avoid cross contamination of specimens and reagents.
- Dispose in accordance with all local and national laws and regulations.

Materials Provided:

Each kit contains material sufficient for 25 sample determinations.

BIO.065.A.25 – Tube containing the Mouse 'Gamma' or 'A' strips (orange arrows strips): 1 Unit.

White plastic tube closed with a desiccant-included cap and grouping twentyfive (25) Mouse Isotyping 'A' lateral flow strips (each with ref. BIO.065.A) for IgG1, IgG2a, IgG2b, IgG2c and IgG3.



BIO.065.B.25 - Tube containing the Mouse 'MuAlKaLa'* or 'B' strips (black arrows strips): 1 Unit.

White plastic tube closed with a desiccant-included cap and grouping twentyfive (25) Mouse Isotyping 'B' lateral flow strips (each with ref. BIO.065.B) for κ , λ, IgA and IgM.

- BIO.065.DB Sample Diluent Buffer: 1 Unit. Vial with 8 mL.
- BIO.065.RL Reading Label: 25 Copies.

Reading labels and transparent patch with covering and self-adhesive capacity for both strips 'A' and 'B'.

*: 'MuAlKaLa' is an acronym for the Greek letters Mu, Alpha, Kappa, and Lambda.

Materials Required But Not Provided:

Microwell plate or test-tube, with diameter greater than 4 mm and preferably with flat bottom and low protein binding.

Specimen Collection And Handling:

No special preparation is required for cell culture / supernatant fluid extraction. No additives or preservatives are required for testing. Use only freshly extracted supernatant fluid.

Experimental Procedure:

- 1. Label all required plate wells or test-tubes with the designation of the sample
- that will be tested. Place the tubes vertically in a tube rack.
 2. Add 100 μ L of Diluent Buffer (Part No. BIO.065.DB) to each well or tube.
 3. Add 100 μ L of sample (purified antibody previously diluted to 1.0 mg/L or freshly extracted supernatant fluid) to the properly labelled well or tube and
- 4. Grasp a single test strip by the written upper part and insert the other end vertically into each well or tube required for testing. Do not immerse the strip too deeply, beyond the line indicated by the arrows (see diagram). Do not swirl or mix once the test strip is added.
- 5. Allow for the solution front to travel the length of the lateral flow strip
- 6. Read results after 10 minutes but no later than 20 minutes, thanks to the reading label (Part Number BIO.065.RL) by positioning the strips on the associated diagrams. Then, place the reading label including the strips as a record in the handling



Interpretation Of Results:

The lines indicating a positive result may develop towards the areas specified by the printing on the reading labels.

Control: a line should develop towards the "control" area. This control line indicates that the test has run properly. If no line develops towards the 'control' area, the test results for that lateral flow test strip are considered uninterpretable and the sample should be re-tested with a new lateral flow strip. Before starting again with another Disp&FLOW® Rapid Isotyping test, it must be ensured that all the instructions for preparation, conservation and implementation of the test have been respected. If all those points were clear, please try the new strip with 10-fold diluted sample.

Positive: a line that develops towards the printing area for the class, subclass, or light chain type indicates the presence of that class, subclass or light chain type in the sample tested.

Negative: If no line develops, except towards the 'control' area, that indicates no mouse antibody is present in the sample tested, or a potential presence at less

Note: Do not interpret results more than 20 minutes after the start of migration.

Limitation Of The Assay:

Results should be used as an aid in determining the classes, subclasses and light chain types present in the sample tested. As with all rapid immunoassays, this method is presumptive. This lateral flow assay is not intended for quantitative analysis. The test procedure and interpretation of the results must be followed closely to obtain reliable results. Any test in which the control line fails to develop is considered indeterminate, and the sample must be re-tested. Each lateral flow test strip is single use only. DO NOT REUSE. The Disp&FLOW® Rapid Isotyping kit is intended for research use only and is not intended for diagnostic, therapeutic, or commercial use.

Detection limit: depending on cell culture /supernatant fluid, it is less than 0.5 mg/L antibodies can be regarded as being the detection limit.

These products are warranted to perform as described in their labelling and in BIOTEM's literature when used in accordance with their instructions. There are no warranties, which extend beyond this expressed warranty, and BIOTEM disclaims any implied warranty of merchantability or warranty of fitness for a particular purpose. BIOTEM's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BIOTEM, to repair or replace the products. In no event shall BIOTEM be liable for any proximate, incidental, or consequential damages in connection with the products.





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