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Accelerated Discovery

Mouse Monoclonal Antibody IgG Subclass Strip Kit

(For Research Use Only. Not for Use in Diagnostic or Therapeutic Applications)

GTX543056



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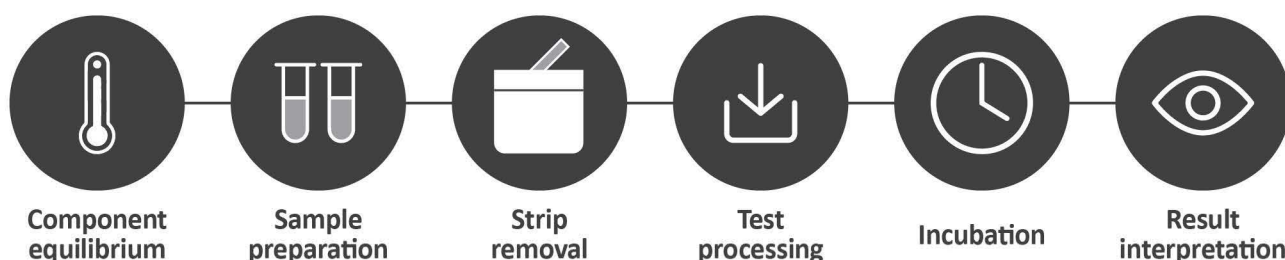
1. Introduction

The GeneTex Mouse Monoclonal Antibody IgG Subclass Strip Kit is a qualitative lateral flow assay developed for the rapid identification of mouse monoclonal antibody IgG subclass. It specifically detects IgG1, IgG2a, IgG2b, IgG3, and kappa light chains through an immunochromatographic method. The schematic diagram (below) gives an overview of the assay procedure.

Each strip contains discrete capture lines corresponding to individual IgG subclasses and the kappa light chain. As illustrated in Figure 1 (page 4), from top to bottom, the visible bands appear in the following order: Control, IgG1, IgG2a, IgG2b, IgG3, and kappa. When a specific subclass is present, a red line will develop at the corresponding location (as well as at the control line, which verifies proper flow and reagent function). This assay does not detect IgG2c or the lambda light chain.

The assay delivers results within 5 to 10 minutes, offering a convenient tool for hybridoma screening or IgG characterization.

Assay procedure



Principle of Detection

The test strip employs a colloidal gold-labeled detection system combined with immobilized IgG subclass-specific capture antibodies printed on a nitrocellulose membrane. When a diluted IgG antibody sample is applied, antibody–gold conjugate complexes are formed and migrate via capillary action. If a detectable subclass is present in the sample, the complex binds to the corresponding capture zone and forms a visible red line.

Note on Light Chains:

This strip specifically detects kappa light chains. Since mouse antibodies possess either kappa or lambda, but not both, a positive result for kappa confirms the light chain identity and indicates the absence of lambda. Therefore, lambda detection is not included to streamline the assay while preserving accuracy.

Advantages

- **Rapid:** Produces results within 5–10 minutes
- **Simple:** No washing or instrumentation required
- **Accurate:** No cross-reactivity between subclasses
- **Efficient:** Designed for high-throughput hybridoma screening
- **Focused:** Targets the most common IgG subclasses and light chain found in mouse monoclonal antibodies
- **Reliable:** Features a positive control line for assay validity

2. Storage Instructions

- Store all components at 2–30°C and avoid direct sunlight.
- Do not freeze.
- Do not reuse strips.
- Do not use components from different kit lots.
- When stored properly, the unopened kit can be stored for up to 12 months.

3. Kit Components

Item	Name	Description	Quantity
A	IgG Subclass Strips	Test strips are individually sealed in pouches containing a desiccant.	10 strips
B	Instruction Manual	Instructions for use	1

4. Additional Materials Required (Not provided)

- 4.1. Pipettes capable of delivering up to a 200 µl volume
- 4.2. Disposable pipette tips suitable for the pipettes indicated above
- 4.3. Development tubes or equivalent sample-loading vessels
- 4.4. Phosphate-buffered saline (PBS) for sample dilution buffer and Negative Control (NC)
- 4.5. Vortex mixer (recommended)

5. Sample Preparation

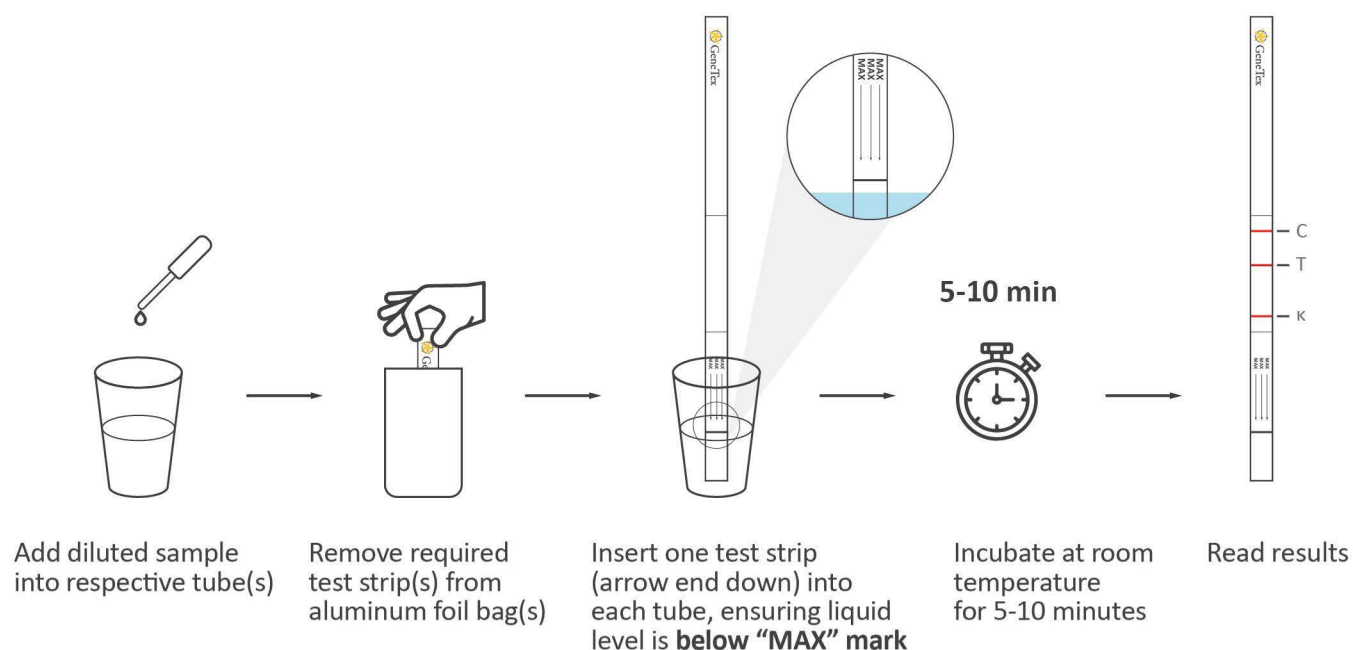
- 5.1. Dilute mouse monoclonal antibody samples to a concentration range of 0.5-10 µg/mL in PBS.
- 5.2. If antibody concentration is unknown, use the following guidelines:
 - **Ascites:** start with a 1:10,000 dilution
 - **Serum*:** start with a 1:15,000 dilution
 - **Culture supernatants:** start with a 1:10–1:100 dilution depending on the culture system
- 5.3. Mix well before use.

*Serum is not recommended due to potential background from host immunoglobulins but may be used with caution.

6. Assay Procedure

The following protocol is for a sandwich immunoassay to detect IgG subclass using a capillary-driven lateral flow strip. Before testing, the antibody sample(s) should be prepared as described above (Section 5). Refer to the scheme shown below for a visual summary of the assay procedure.

Sample Preparation



Step-by-Step Instructions:

- 6.1. Allow all kit components to equilibrate to room temperature (18–25°C) before use. This prevents moisture condensation that may interfere with assay performance.
- 6.2. Remove the required number of test strips and development tubes (not provided).
- 6.3. Pipette 75 μL of the prepared negative control into a tube labeled “NC” (if needed). For each test sample, pipette 75 μL of the diluted antibody sample into a separate tube.
- 6.4. Insert one test strip into each tube with the arrow end pointing downward.
⚠ IMPORTANT: Ensure that the total liquid volume is not above the black line beneath the “MAX” arrow marking on the strip (see Figure 1). This line indicates the maximum allowable liquid level for proper capillary flow.
- 6.5. Allow the test strips to develop at room temperature for 5–10 minutes.
- 6.6. Once the red control band appears on the strip, read and interpret the result.

7. Interpretation of Results

7.1. Figure 1 presents a 1:1 scale layout of the GeneTex Mouse Monoclonal Antibody IgG Subclass Strip. The strip includes the following features:

- C: Control line – Verifies the assay is working properly
- T: Test zone – Consists of individual bands for each subclass: IgG1, IgG2a, IgG2b, and IgG3
- κ: Kappa line – Indicates the presence of a kappa light chain

Users can place their used test strip next to the image in Figure 1 to visually compare the band positions. This allows for straightforward identification of the subclass(es) present in the sample based on which bands are visible.

- 7.2. A red line in the control region indicates the strip is functioning properly.
- 7.3. If only the control line is detected and no other band appears, the assay is negative (see diagram below and Section 8: Troubleshooting Guide).
- 7.4. Red bands appearing on both the control line (C line) and one or more of the test lines means a positive result.
- 7.5. Appearance of one or more red lines at specific subclass positions (Test zone) and/or kappa (κ) line indicates the presence of the corresponding immunoglobulin subclass(es). The table below shows examples of possible result readouts.

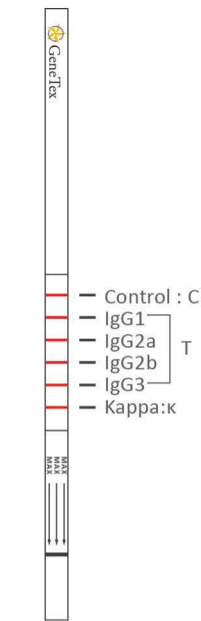


Figure 1

Result		Representative illustration
Valid	Negative	
	Positive	
Invalid		

* C: Control, T: IgG1, IgG2a, IgG2b, IgG3, κ: Kappa

Figure 1. Actual-size reference image of the Mouse Monoclonal Antibody IgG Subclass Strip.
*For optimal comparison, ensure proper printing settings.
*Note: To ensure Figure 1 appears at actual size (1:1 scale), please print on **A4 paper** with **scaling disabled** (e.g., "fit to page" turned off) and using **one page per sheet**.

7.6. Examples

- If red bands appear at the IgG1 and kappa positions, the IgG subclass is IgG1-kappa.
- If only the control band appears, the sample contains no detectable IgG1/2a/2b/3 or kappa.

8. Troubleshooting Guide

While the assay is designed for robust performance, variability in antibody structure or sample composition may affect outcomes. The analysis might be non-specific, and efficiency can vary from antibody to antibody. The standard protocol may not work for every test or antibody of interest. We highlighted some frequently encountered problems and their potential causes and solutions (see table below and **Section 9. Notes**).

Problem	Possible Cause	Solution
No test band appears	Antibody too dilute or absent	Use higher concentration; confirm antibody is mouse-derived
Multiple bands	Sample contamination, mixed hybridomas, or overconcentrated sample	Further dilute sample; ensure monoclonality
No control band	Insufficient sample volume or premature strip removal	Retest with adequate volume applied and allow 10 minutes for development

9. Notes

Sample Compatibility and Limitations

- Samples compatible with this kit include hybridoma culture, purified antibody, diluted ascites, and serum samples.
- Not suitable for detecting IgM, IgA, IgD, IgE, IgG2c, or lambda light chain.
- Mild cross-reactivity may occur with IgG2a in rare cases.

Factors That May Affect Test Performance

- Sample matrix (e.g., buffer composition) may affect flow rate and signal intensity.
- Environmental and operational factors (e.g., temperature, humidity, incubation time) may cause minor variations in specificity and affect test performance.

Operational Recommendations

- For optimal consistency, always use freshly prepared dilutions.
- Do not wash the strip during or after development.

Reading Time Guidelines

- Read time: 5–10 minutes. In some cases (e.g., when testing very dilute samples), the test may take up to 15 minutes.
- Do not interpret results after 20 minutes.