INSTRUCTIONS

Pierce Rapid Isotyping Kits – Mouse

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Number

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Description Pierce Rapid Isotyping Kit – Mouse, contains sufficient reagents for 10 samples containing mouse IgG₁, IgG_{2a}, IgG_{2b}, IgG₃, IgA and IgM Pierce Rapid Isotyping Kit also Korne and Lambda. Mouse contains sufficient reagents for 10

Pierce Rapid Isotyping Kit plus Kappa and Lambda – Mouse, contains sufficient reagents for 10 samples containing mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM and kappa and lambda light chains

Kit Contents:

Isotyping Cassettes, 10 pouches containing two or three cassettes. The standard kit contains a cassette with a strip impregnated with anti-IgG₁, -IgG_{2a} and -IgG_{2b} antibodies and a cassette with a strip impregnated with anti-IgG₃, -IgA and -IgM antibodies. The kappa and lambda kit includes a cassette impregnated with anti-kappa and -lambda antibodies. Each cassette contains a control band.

Sample Diluent, 45mL

Storage: Upon receipt store kit at 2-8°C.

Introduction

The Thermo ScientificTM PierceTM Rapid Isotyping Kit is a 5-minute lateral-flow assay with ELISA sensitivity for class and subclass determination of monoclonal antibodies. The assay is performed by simply adding a properly diluted tissue culture supernatant or mouse ascites sample to the well of the cassette. The gold conjugates embedded in the cassette form specific class- and subclass-soluble complexes with the antibodies in the sample. These complexes travel the length of the membrane and are resolved on the anti-isotype and class-specific antibody-impregnated membrane. Results are displayed as a red band indicating the antibody isotype or subclass.

Material Preparation

Note: proper sample dilution is essential for optimal results.

Mouse ascites fluid	Dilute ascites 1:8000 by adding 0.5μ L of ascites fluid to 4mL of Sample Diluent and vortex to mix. If sample concentration is known, dilute to 100ng/mL.	
Cell/tissue culture supernatant	Dilute cell culture/supernatant fluid 1:100 by adding 5μ L of supernatant to 0.5mL of Sample Diluent and vortex to mix.	
	For supernatants containing $< 1\mu$ g/mL monoclonal antibody, dilute sample 1:10 by adding 50µL of supernatant to 450µL of Sample Diluent and vortex to mix.	

Procedure

- 1. Before opening a pouch of cassettes to be used, remove it from refrigeration and equilibrate it fully to room temperature.
- 2. Open the pouch containing the cassettes and add 150µL of diluted sample to the well of each cassette.
- 3. Wait 5-10 minutes for color bands to appear and immediately evaluate results.
- 4. A successful test will result in a red band at the "C" or control location and a darker band at one of the three isotypes on each cassette. The darker line is the isotype.

Note: Additional weaker red lines might appear, indicating the presence of host serum immunoglobulins or multiple hybridoma clones.



Troubleshooting

Problem	Possible Cause	Solution
No bands detected after applying sample	Sample did not completely enter the sample well	Gently agitate cassette to initiate movement of the sample onto the test strip
	Sample does not contain antibody	Verify sample contains antibody by another test (e.g., ELISA or Western blot)
Multiple bands detected	Sample not sufficiently diluted	Dilute sample an additional 1:10 to 1:100 and retest
	Sample contains antibody from multiple sources	Dilute sample an additional 1:10 to 1:100 and retest (the darkest band is the isotype of the most abundant antibody in the sample)
	Myeloma cell line used in hybridoma production secretes immunoglobulin	
	The test was allowed to proceed longer than 10 minutes	Evaluate results within 10 minutes of adding the sample to the cassette well

Related Thermo Scientific Products

37503	Pierce Rapid ELISA Mouse mAb Isotyping Kit
37501	Monoclonal Antibody Isotyping Kit (HRP/ABTS)
23300	Easy-Titer Mouse IgG Assay Kit
23305	Easy-Titer Rabbit IgG Assay Kit
23310	Easy-Titer Human IgG (H+L) Assay Kit
23325	Easy-Titer Human IgG (gamma chain) Assay Kit
23315	Easy-Titer Human IgM Assay Kit
22810	Pierce Protein A Plus, 1mL
22851	Pierce Protein G Plus, 2mL
20520	Pierce Protein L Plus, 2mL
20423	Pierce Protein A/G Plus, 2mL

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