DRG® Mouse Isotyping Test (RAP-4461)

REVISED 16 AUG. 2005 **RUO**

**PRODUCTS ARE FOR RESEARCH USE ONLY,
AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE.**

1 KIT CONTENTS

- 1 Desiccant vial containing mouse isotyping test strips, 10 tests.
- 10 Capped ready to use lyophilized microparticle development tubes.

2 TEST PRINCIPLE

The assay principle is based on anti-mouse kappa and anti-mouse lambda antibodies coupled onto coloured micro particles and equally reactive to any mouse monoclonal antibody regardless of its isotype.
The isotyping strip has immobilized bands of goat anti-mouse antibodies corresponding to each of the common mouse antibody isotypes (IgG1, IgG2a, Ig2Gb, IgG3, IgM, and IgA) and to the kappa and lambda light chains.
Both sides of the strip bear a positive flow control band, which indicates that the antibody-coated coloured micro particles have migrated through the strip.
By 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 where it forms a complex with the antibody coated micro particles, and inserting the strip.
This complex flows through the strip until it is bound by the immobilized goat anti-mouse antibody specific for the monoclonal's isotype and it's light chain.
In approximately 5-10 minutes, the micro particle complex will aggregate as blue bands in the two sections corresponding to the monoclonal antibody's isotype and its light chain.
Development of the strip is complete when the positive flow control band on each side of the test strip turns blue.

3 SPECIFICITY

The mouse monoclonal antibody isotyping test kit shows no cross-reactivity with bovine-IgG (<0.1 %).

4 PROCEDURE

Note: All reagents should be brought to room temperature before use.

4.1 Sample Preparation

Dilute all monoclonal antibody samples to a concentration of 1.0µg/ml in PBS.
If the concentration of the sample is entirely unknown, make dilutions based on the following estimates.

Typically, serum contains between 10-15mg/ml IgG, ascites can be as high as 10mg/ml and cell culture supernatants usually contain 0.5-1.0mg/ml.
Using these estimates, the appropriate dilutions can be made.

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

4.2 Assay Protocol

1. Remove the desired number of isotyping strips from the desiccant vial and recap.
   Remove the caps from an equal number of development tubes.
   Note: the tubes may be labeled with a marker for identification.
2. Pipette 150ul of the freshly diluted sample into each development tube and
   incubate at room temperature for 30 seconds.
   Vortex the tube briefly to ensure that the coloured micro particle solution is completely re-suspended.
3. Place one isotyping strip, with the solid red end at the bottom, in each development tube.

5 INTERPRETATION OF RESULTS

Interpret the results at 5-10 minutes once the positive flow control bands have appeared.

Within 5-10 minutes, a blue band will appear above the letters in one of the class or subclass windows as well as in either the kappa or lambda window of the strip, indicating the heavy and light-chain composition of the monoclonal antibody.

The intensity of the blue bands will increase as the sample continues to flow up the strip.

The positive flow control bands on each side of the isotyping test strip should also appear, indicating that the antibody-coated micro particles are functional and have flowed up the strip.

In cases where the sample is very dilute, the development time may take up to 10 minutes.

Note:
For a permanent experimental record or for an easier interpretation of results when testing multiple samples, the solid red area may be cut off the bottom of the strip to prevent further band development once the positive flow control bands have appeared.
A gentle stream of air could be applied to the membrane portion of the strip to assist in drying the membrane and preventing any further development.

Do not wash the strip to stop the reaction.

6 TROUBLESHOOTING

Problem: No heavy and or light-chain band appeared on the strip, but the positive flow control bands appeared.

POSSIBLE REASON	RECOMMENDATION
The antibody concentration was too low	Prepare a less-dilute sample and retest
No antibody was in the sample	The hybridoma is either not secreting. If possible sub-clone the hybridoma and retest.
Freshly diluted samples were not used	Prepare fresh dilutions and retest

Problem: Multiple heavy and light-chain bands appear on the strip.

POSSIBLE REASON	RECOMMENDATION
Antibody concentration was too high	Prepare a less-dilute sample and retest
For ascites, there may be small amounts of contaminating antibodies produced	Dilute sample further and retest.
For tissue culture supernatant, a mixed culture may be present	Re-clone the hybridoma and re-test

Problem: No positive flow control bands appear:

POSSIBLE REASON	RECOMMENDATION
Sample volume was too low (<150µl).	Carefully dilute a fresh sample and pipette 150µl into a new development tube and retest
Strip removed from development tube too early	Retest and allow strip to react for at least 10 minutes

7 STORAGE AND STABILITY

The kit contents are stable for 12 months after the date of despatch when stored at +2 to +8°C.
Do not use components from different lots.
DO NOT FREEZE.

SYMBOLS USED WITH DRG ELISA´S

Symbol	English	Deutsch	Francais	Espanol	Italiano
	User´s Manual	Arbeitsanleitung	Mode d'emploi	Instrucciones de empleo	Istruzioni d'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke			
REF	Catalogue number	Katalog-Nr.	Référence	No de catálogo	No. di Cat.
LOT	Lot. No.	Chargen-Nr.	No. de lot	Número de lote	Lotto no
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Instruções de uso	Brugermanual	Användar manual	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..