TEST KIT CONTENTS

20 Test Kit	Description
10 Packs	Ready-to-use Twin DEVICES.
10 Vials	Lyophilized Goat anti-mouse Colloidal Gold CONJUGATE*.
5 ml	DILUENT
2.5 ml	PREWET (BUFFER)
10 ml	CLEAR SOLUTION

* Reconstitute lyophilized Conjugate with 200 µl DILUENT prior to use.

EY Laboratories, Inc.
107 N. Amphlett Blvd. San Mateo CA 94401 USA
Revision (06/03)

Instant CHEK™ ONE-MINUTE ISOTYPING KIT

For monoclonal antibodies
Cat. No.: IC-IS-002-20

INTRODUCTION

Traditionally, isotyping of mouse monoclonal antibodies is performed using an antigen-capture ELISA, a slow procedure that takes hours to perform. In addition, the amount of hybridoma supernatant required often limits isotyping during the 96 well phase of hybridoma growth. For many reasons it is desirable to know the isotype of monoclonal antibodies early in the selection procedure in order to eliminate undesirable hybridomas.

The ***Instant* CHEK™ ONE-MINUTE ISOTYPING KIT** requires only a few microliters of hybridoma supernatant and provides unequivocal results before other assays have even started.

ASSAY PRINCIPLE

The *Instant* CHEK™ rapid assay technology is a proprietary method based on *Immunofiltration affinity chromatography.*

Each *Instant* CHEK™ isotyping device contains five different goat anti-mouse isotype-specific antibodies immobilized at five different positions on a proprietary porous membrane (diagram 1). A patented directional absorbent system allows concentration of mouse monoclonal antibodies onto the capture antibodies thus allowing high sensitivity and specificity (diagram 2).

Diagram 1
Localization of anti- isotype Capture Antibodies on device:

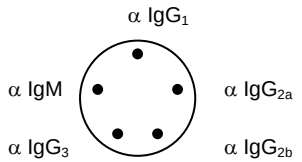
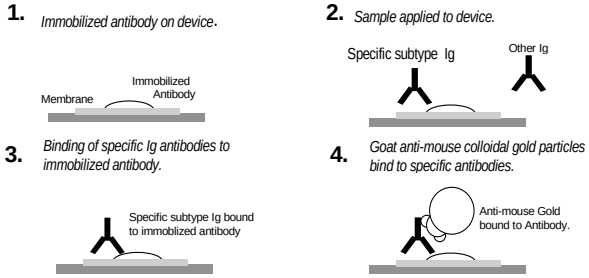


Diagram 2
Principle of Methodology:



SPECIFICITY

This kit is designed for isotyping of mouse monoclonal antibodies of IgM, IgG_1, IgG_{2a}, IgG_{2b} and IgG_3 classes.

For determination of IgA, IgD and kappa and lambda chains please contact EY Laboratories technical services.

SENSITIVITY

Mouse subclass antibody detection is 1μg/ml or less, equivalent to ELISA.

PROTOCOL

1. At least **10 minutes** prior to use, for each 2 tests, reconstitute 1 vial of lyophilized GOLD CONJUGATE with 200 μl of DILUENT.
2. Use 100 μl of undiluted hybridoma tissue culture supernatant as SAMPLE. (If the sample has been diluted with PBS or water, suggest mix 100 μl of undiluted hybridoma tissue culture supernatant with 100μl of DILUENT and mix well before add onto the membrane.)
3. ASSAY PROCEDURE (use one *Instant* CHEK™ Device per hybridoma supernatant).

   Step 1 Add 2 drops of PREWET to wet the membrane. Wait until absorbed.

   Step 2 Use 100 μl of undiluted hybridoma tissue culture supernatant as SAMPLE. (If the sample has been diluted with PBS or water, suggest mix 100 μl of undiluted hybridoma tissue culture supernatant with 100μl of DILUENT and mix well before add onto the membrane.) Add 100μl undiluted sample or 200 μl of diluted SAMPLE (diluted culture supernatant) onto the membrane. Wait until absorbed

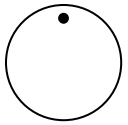
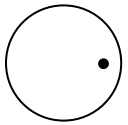
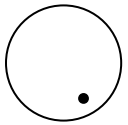
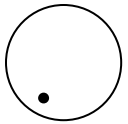
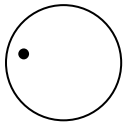
   Step 3 Add 2 drops (80 μl) of reconstituted GOLD CONJUGATE. Wait until absorbed.

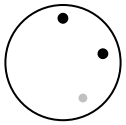
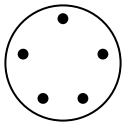
   Step 4 Add 4 drops of CLEAR SOLUTION and read results. If a pink background still appears, add additional drops of CLEAR SOLUTION to wash away.

- After completed the assay, peel off top of device with membrane to avoid back diffusion. Attach to notebook for permanent results.

NOTE: Once reconstituted, each vial of GOLD CONJUGATE contains enough solution for two (2) tests.

OBSERVED REACTIONS

OBSERVATION	INTERPRETATION
	IgG_1
	IgG_{2a}
	IgG_{2b}
	IgG_3
	IgM

OTHER EXAMPLES	INTERPRETATION
	Strong IgG_1 Strong IgG_{2a} Weak IgG_{2b} (Several isotypes present)
	IgG_1 IgG_{2a} IgG_{2b} IgG_3 IgM (All isotypes present)
	High Background Dilute sample or filter, test on another device

CAUTIONS AND LIMITATIONS

a) Samples containing monoclonal antibodies must be of good quality. If ascites fluids are used (not recommended), dilute 1:100 (or more) in DILUENT (provided in kit). Take care to clarify samples and to remove lipids or particulate matter. If antibodies are at high concentrations false positive reactions may occur.

b) Hybridoma supernatant samples should be from several day old cultures so that antibody has time to accumulate. If antibodies are in sufficient concentration (1 μg/ml or less) but amount of supernatant is limiting, then increase volume by addition of DILUENT. Diluted sample should be added in a total volume of 200 μl.