

MOUSE MONOCLONAL ANTIBODY ISOTYPING KIT
10 ASSAY KIT (DIPSTICK FORMAT)

Storage conditions: 2 – 4 °C. Do not freeze.

This product is distributed for research use only.

It has not been approved for diagnostic or drug purposes or for administration to humans or animals.

INTRODUCTION

The Hycult Biotech Mouse Monoclonal Antibody Isotyping Kit (Dipstick Format) is based on a detection system which eliminates the needs for radioactive tracers, enzymes and substrates and reduces the number of handling steps.

The one-step procedure involves the capture of the mouse immunoglobulins by subclass specific rat anti-mouse monoclonal antibodies which are immobilized on the test strip. Captured mouse immunoglobulins are detected directly by a second rat monoclonal antibody which is coupled to colloid particles. This detector complex results in the formation of two coloured spots (one isotype and light chain) on the test strip. Results are visible in as little as 30 minutes depending on the concentration of immunoglobulin in the test sample. The bound antibody and colloidal detector complex form a visual signal and a permanent record of results. No stopping reaction is required and incubation periods of longer duration (even during 8 hours) will increase the signal without generating higher background values.

NOTE:

Less than 2% of hybridoma lines produce immunoglobulins which consist of a λ light chain. Such antibodies can be detected in the λ isotyping procedure.

CONTENTS OF THE KIT

Item no.

- 1: 1 Vial colloidal rat anti mouse κ conjugate (10 ml). Preservative: 2-chloroacetamide.
- 2: 1 Vial dilution buffer (15 ml). Preservative: 2-chloroacetamide.
- 3: 1 Vial colloidal rat anti mouse λ conjugate (1 ml). Preservative: 2-chloroacetamide.
- 4: 1 Stick for λ isotyping (prepacked in test tube).
- 5: 10 Sticks for κ isotyping (prepacked in test tubes).
- 6: Rack for test tubes
- 7: Protocol
- 8: Datasheet

APPLICATIONS

Potential uses of this kit are as follows:

- Isotyping mouse antibodies
- Controlling cell lines for isotype switching
- Selecting monoclonal antibodies on the basis of isotype
- Confirming cessation of antibody production by hybridomas
- Detecting and identifying bifunctional antibodies
- Process control during antibody purification

WARNINGS, LIMITATIONS AND PRECAUTIONS

- Do not mix reagents from different master lots.
- Several components in the product are preserved using 2-chloroacetamide. All of these may be hazardous to health and direct contact with skin, eyes etc., should therefore be minimized by careful handling. The use of gloves during the handling of these substances is strongly recommended.

STORAGE INSTRUCTIONS

- Upon receipt, store kit at 2 – 8°C. **DO NOT FREEZE**. Do not use components beyond the expiration date printed on the label.
- All reagents should be brought to room temperature (18 – 25°C) prior to use and should be stored at 2 – 8°C immediately after use.

DIRECTIONS FOR USE

Optimal detection is achieved at antibody concentrations in the range of 1 - 50 $\mu\text{g/ml}$. Due to the nature of the test, high antibody concentrations > 50 $\mu\text{g/ml}$ can produce a decreased signal which is caused by saturation of the antibody binding site on the detector complex.

Tissue culture supernatant

Monoclonal antibody concentrations in culture supernatant are usually within this range but there are cell culture techniques (i.e. hollow fibre, fermenter) which produce antibody at concentrations >200 $\mu\text{g/ml}$. Also the antibody concentration in concentrated tissue culture supernatant and (affinity) purified antibody fractions can be much higher. In cases where the antibody concentration is known to be higher than 50 $\mu\text{g/ml}$, a dilution is necessary.

The dilution can be made in any saline buffer or culture medium.

Ascites

In ascites the antibody concentration can vary between 1-20 mg/ml. Up to 50% of these antibodies may be host antibodies (polyclonal). Generally, a dilution of 100-1,000 times prevents the appearance of more than two spots. Sometimes however, a dilution of 10,000 times is necessary in order to prevent this problem. For a proper subclass determination, culture supernatant is recommended. Dilutions can be made in a saline buffer (e.g. PBS; 0.096% (w/v) $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 0.0175% (w/v) KH_2PO_4 0.9% (w/v) NaCl, pH 7.4).

κ ISOTYPING PROCEDURE

1. Add 500 μl buffer to a test tube containing a dipstick for κ isotyping.
2. Add 500 μl sample (culture supernatant or diluted ascites fluid) to the test tube.
3. Submerge dipstick completely by gentle agitation.
4. Resuspend the rat anti-mouse κ conjugate by shaking the bottle and add 1 ml to the test tube containing the dipstick ensuring that the printed side is facing down.
5. Agitate at 18-25°C until two spots show up (one isotype and κ). The dipstick (printed side facing down) should remain in contact with the solution during agitation.

Notes:

- Intensive coloured spots can be obtained by prolonging the incubation period (maximal 8 hours) providing that sufficient concentration of antibody is present.
- Any method of gently agitating is acceptable.
- Normally the spots develop between 30 and 60 minutes, however, if no spots develop after two hours, antibody is of the λ isotype or alternatively antibody concentration is too low or far too high. If concentration is within the right range, proceed with λ isotyping procedure. If the antibody concentration is probably too high, try other dilutions.

λ ISOTYPING PROCEDURE

1. Add 500 μ l buffer to a test tube containing a dipstick for λ isotyping.
2. Add 500 μ l sample (culture supernatant or diluted ascites fluid) to the test tube.
3. Submerge dipstick completely by gentle agitation.
4. Resuspend the rat anti-mouse λ conjugate by shaking the bottle and add 1 ml to the test tube containing the dipstick ensuring that the printed side is facing down.
5. Agitate at 18-25°C until two spots show up (one isotype and λ). The dipstick (printed side facing down) should remain in contact with the solution during agitation.

Problem

No spots developing on stick after κ isotyping procedure.

No spots developing on stick after λ isotyping procedure.

Weak spot developing on sticks.

Only isotype spot shows. κ/λ spots are not visible.

More than two spots develop.

TROUBLE SHOOTING GUIDE

Possible Cause

1. Concentration of antibody is too low.
2. The antibody has a λ light chain.
3. Concentration of antibody in sample is far too high.
1. The antibody is degraded and the fragments are blocking the reaction i.e. "sandwich" formed with the detector complex since this is no longer possible.
2. Concentration of antibody in test sample is too low.
3. Improper storage of the kit.
4. Improper use of kit reagents
1. Low concentration of antibody in test sample.
1. The test results is due to the fact that the sensitivity of detection of the heavy chains is at least twice of that of the light chains.
1. Culture supernatant contains more than one antibody.
2. In the case of ascites fluid, host antibodies might be present.
3. Isotyping switch variants might be present in the sample.

Remedy

1. Add more (concentrated) sample. Incubate for at least 4 - 8 hours.
2. Proceed with λ isotyping procedure.
3. Dilute the sample and repeat the assay.
1. Take a freshly prepared sample.
2. Add more (concentrated) sample and incubate overnight.
3. Check storage conditions.
4. Check the procedure.
- 1a. Add more (concentrated) sample and incubate overnight.
- 1b. Prolong incubation period.
- 1c. Pour off the antibody-conjugate mixture and add dilution buffer and rat anti-mouse κ only.
- 1a. The conjugate used determines the light chain. Repetition of the assay is not necessary.
- 1b. Prolong the incubation period.
1. Subclone your cell line.
2. Dilute ascites fluid further until only two spots show up (1 isotype plus κ or λ).
3. See section on isotype switch variant.

ADDITIONAL INFORMATION

Isotype Switch Variants

One clone of a hybridoma cell line should produce antibodies belonging to one subclass only. However because of gene rearrangements some cells may switch to produce a different subclass. The order of C heavy genes (5'-3') has been identified as μ , δ , $\gamma 3$, $\gamma 1$, $\gamma 2b$, $\gamma 2a$, ϵ and α . Isotype switching has been reported, except for μ (IgM), to be unidirectional from 5'-3'. This means that some cells of a clone producing for instance IgG, may suddenly produce IgG2b, but never IgG3. The frequency of switching differs with culture time but can vary between less than 1:400,000 producing cells to more than 1:2,000 in the latter case, meaning that of very 106 cells, 500 cells produce "next" isotype immunoglobulins. Sensitive assays can detect the presence of these "contaminants".

Subclass	$\gamma 3$	$\gamma 1$	$\gamma 2b$	$\gamma 2a$	ϵ	α
Possible major contaminant	$\gamma 1$	$\gamma 2b$	$\gamma 2a$	ϵ	α	-
Possible minor contaminant	$\gamma 2b$	$\gamma 2a$	ϵ	α	-	-

REFERENCES

1. Boot, JH et al; Murine monoclonal isotype switch variants. Detection with rat monoclonal antibodies in ELISA and isolation by sequential sublining. J Immunol Methods 1988, 106: 195
2. Esser, C et al; Immunoglobulin class switching: molecular and cellular analysis. Annu Rev Immunol 1990, 8 : 717