USER GUIDE

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Fluorescein Isothiocyanate (FITC)-conjugated Antibodies

Catalog Numbers R933-25, R953-25, R963-25

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For Research Use Only. Not for use in diagnostic procedures.

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Contents

Important Information

Types of Antibodies

This manual is supplied with the fluorescein isothiocyanate (FITC)-conjugated antibodies listed in the following table.

Antibody	Catalog No.
Anti-His(C-term)-FITC	R933-25
Anti-*myc*-FITC Antibody	R953-25
Anti-V5-FITC Antibody	R963-25

Shipping/Storage

Each FITC-conjugated antibody is shipped in an amber vial and should be stored at 4°C protected from exposure to light. Each product is guaranteed for six months from the date of receipt if properly stored.

For long-term storage, aliquot the antibody and store at –20°C or –80°C protected from exposure to light. Repeated freezing and thawing is not recommended as it may result in loss of antibody activity.

WARNING! GENERAL CHEMICAL HANDLING. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Contents

Each FITC-conjugated mouse monoclonal antibody is supplied in Phosphate-Buffered Saline (PBS) and 0.01% sodium azide (added as a preservative). The following table provides information on the concentration of antibody, amount supplied, and the antibody subclass for each FITC-conjugated antibody. The amount of antibody provided is sufficient for 25 immunostaining reactions using a 1 mL working solution per reaction.

Antibody	Concentration	Amount	Subclass
Anti-His(C-term)-FITC	1 mg/mL	50 µl (25 reactions)†	IgG_{2b}
Anti-*myc*-FITC	1 mg/mL	50 µl (25 reactions)†	IgG_1
Anti-V5-FITC	1 mg/mL	50 µl (25 reactions)†	IgG_{2a}

†Assumes 1 mL working solution per immunostaining reaction to give a final antibody concentration of 2 µg/mL

Antibody Conjugation

The Anti-His(C-term), Anti-*myc*, and Anti-V5 FITC-conjugated antibodies were prepared by crosslinking the appropriate primary antibody with the FITC fluorophore using established protocols (Harlow and Lane, 1988).

Methods

Overview

Introduction

The Anti-His(C-term)-FITC, Anti-*myc*-FITC, and Anti-V5-FITC antibodies allow immunofluorescence detection of recombinant fusions proteins containing the His(C-term), *c-myc*, and V5 epitope tags, respectively. Many of the expression vectors available from Life Technologies contain one or more of these epitope tags. The FITC-conjugated antibodies may also be used to detect recombinant proteins expressed from vectors sold by other manufacturers (if they contain the appropriate epitope tag). For more information about the various expression vectors available from Life Technologies visit **www.lifetechnologies.com** or call Technical Support (see page 8).

Epitope

The following table describes the epitope recognized by each FITC-conjugated antibody.

Product	Epitope	Amino Acid Sequence of Epitope
Anti-His(C-term)-FITC Antibody	Detects fusion proteins containing a C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection (Lindner *et al.*, 1997)	His-His-His-His-His-His-COOH
Anti-*myc*-FITC Antibody	Detects fusion proteins containing a 10 amino acid epitope derived from *c-myc* (Evan *et al.*, 1985)	Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu
Anti-V5-FITC Antibody	Detects fusion proteins containing a 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991)	Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr

Antibody Specificity

All FITC-conjugated antibodies have been tested in immunofluorescence experiments using cultured CHO (Chinese Hamster ovary) cells expressing recombinant epitope-tagged fusion proteins. Low background was observed using the protocol on page 2. The FITC-conjugated antibodies have also been tested in immunoblotting procedures with purified Positope™ control protein.

Important

Do not expose the FITC-conjugated antibody to light. Continuous exposure to light will cause the FITC-conjugated antibody to gradually lose its fluorescence.

Recommended Dilutions

For immunofluorescence on mammalian cells, we recommend diluting the FITC-conjugated antibody 1:500 into Phosphate-Buffered Saline (PBS) containing 10% fetal bovine serum (FBS).

Note: Depending upon your particular application, sample type, or cell line, you may want to determine empirically the appropriate dilution of antibody to use in your experiments.

Immunofluorescence

Introduction

Many protocols can be used for detection of recombinant fusion proteins with the appropriate FITC-conjugated antibody. The following general protocol for performing immunofluorescence on cultured cells is included for your convenience. Other protocols are suitable. For details and other protocols, please refer to published reference sources (Ausubel *et al.*, 1994; Harlow and Lane, 1988).

Reagents and Equipment Required

The following materials and solutions are needed for immunofluorescence:

- Phosphate-Buffered Saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \bullet 7H_2O$, 1.4 mM KH_2PO_4, pH 7.3)
- Fetal Bovine Serum (FBS)
- Methanol
- Blocking buffer (PBS + 10% FBS, w/v)
- Appropriate FITC-conjugated antibody
- Fluorescence microscope
- FITC filter or other appropriate filter

Performing Immuno-fluorescence on Cultured Cells

The following protocol may be used to perform immunofluorescence on cells plated in a 35-mm tissue culture dish or a single well in a six-well tissue culture plate. Please note that volumes may need to be adjusted if you are using larger or smaller plates.

1. Plate cells in a 35-mm dish or a single well in a six-well tissue culture plate. Incubate cells overnight at 37°C in serum-containing medium or until they reach 50% confluence.
2. Transfect cells with the expression construct of choice and allow an appropriate amount of time to elapse for expression of your recombinant fusion protein containing the epitope tag.
3. Remove the medium and wash cells twice with PBS.
4. Fix the cells by adding 2 mL of room temperature, 100% methanol.
   Note: Depending on the nature of the protein being detected, other fixatives may be used. Some empirical experimentation may be necessary.
5. Incubate for 5 minutes at room temperature. Do not exceed 5 minutes.
6. After incubation, wash cells 5 × 2 minutes with PBS (2 mL/wash).
7. Add 2 mL of blocking solution (PBS containing 10% fetal bovine serum [FBS]) and incubate for 20 minutes at room temperature to reduce non-specific binding of antibody.
8. Remove the blocking solution and add 1 mL of PBS/10% FBS containing the appropriate FITC-conjugated antibody (1:500 dilution of antibody). Incubate for 1 hour at room temperature in the dark.
9. Wash cells 2 × 5 minutes with PBS and observe cells with a fluorescence microscope equipped with a FITC filter (or appropriate filter).

Troubleshooting

The following table lists some potential problems and possible solutions that you may use to help you troubleshoot your immunofluorescence experiment.

Problem	Reason	Solution
No signal	Little or no fusion protein expression	Repeat transfection and expression. Harvest cells and use western blot analysis to check for fusion protein expression (see **Note** immediately following).
	Antibody too dilute	Use more antibody.
	Poor fixation	Try alternative fixation methods.
High background	Antibody too concentrated	Titrate the antibody and use the maximal dilution that gives a detectable signal in a reasonable amount of time.
	Insufficient blocking	Increase incubation time in blocking solution.

Note

Each FITC-conjugated antibody is also suitable for use in western blot analysis to detect expression of recombinant fusion proteins containing the appropriate epitope tag. Use the FITC-conjugated antibody to probe your western blot, then perform secondary detection using a HRP- or AP-conjugated secondary antibody.

Appendix

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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Notes

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