For life science research only.
Not for use in diagnostic procedures.

Anti-HA-Fluorescein, High Affinity

from rat IgG_1 (clone BMG 3F10)

Version: 08

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Monoclonal antibody for the highly sensitive detection of HA-tagged recombinant proteins, F_{ab} fragments conjugated with fluorescein.

Cat. No. 11 988 506 001 25 µg

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Anti-HA-Fluorescein, High Affinity (3F10)	▪ White lyophilizate ▪ Lyophilized in the presence of proteinous stabilizers.	1 vial, 25 µg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Anti-HA-Fluorescein, High Affinity (3F10)	Store at +2 to +8°C.

Reconstitution

1. Add 0.5 ml double-distilled water to the lyophilizate to a final concentration of 50 µg/ml. Let stand for at least 10 minutes at +15 to +25°C.
2. Mix thoroughly; do not vortex.
3. Store 2 months at +2 to +8°C or aliquot and store 6 months at −15 to −25°C.

⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For preparation of lyophilizate

- Double-distilled water

For flow cytometry and fluorescence microscopy

See section, **Working Solution** *for additional information on preparing solutions.*

- PBS*
- FCS or rat serum
- Standard flow cytometer
- Fluorescence microscope

1.4. Application

Anti-HA-Fluorescein is used for:

- Flow cytometry
- Immunofluorescence applications

2. How to Use this Product

2.1. Before you Begin

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Working Solution	Composition or preparation	Storage and Stability	For use in...
Phosphate buffered saline (PBS)*, 10x	100 mM phosphate, 1.5 M NaCl, pH 7.2	Store 1 week at +2 to +8°C or at least 2 years at −15 to −25°C.	Preparation of 1x PBS.
PBS, 1x	Dilute 10 ml 10x PBS with double-distilled water to a final volume of 100 ml.		▪ Preparation of Washing buffer ▪ Anti-HA-Fluorescein labeling solution
Incubation buffer	1x PBS, containing 1 to 5% FCS and/or rat serum.	Unstable, prepare immediately before use.	Preparation of Anti-HA-Fluorescein solution
Anti-HA-Fluorescein labeling solution, 2x	Dilute the reconstituted antibody to 2 – 10 µg/ml in Incubation buffer.	Unstable, prepare immediately before use.	Detection

2.2. Protocols

Flow cytometry and fluorescence microscopy

The following protocol describes the detection of a HA-tagged protein by flow cytometry and fluorescence microscopy using a standard flow cytometer and a fluorescence microscope.

Staining procedure

See section, **Working Solution** *for additional information on preparing solutions.*

1. Wash 10^6 to 10^7 cells expressing an HA-tagged fusion protein with Incubation buffer.
   – Centrifuge the cells at 400 × *g* for 10 minutes.
   If the HA-epitope of the tagged protein is expected to be expressed intracellular, permeabilize the cellular membrane before staining.

2. Resuspend the cells in Incubation buffer at a concentration of 1 × 10^7 cells/ml.
   – Incubate at +2 to +8°C for 15 minutes.
   – During incubation, prepare the Anti-HA-Fluorescein labeling solution, 2x conc.

3. Mix equal volumes, for example, 50 µl of the cell suspension with Anti-HA-Fluorescein labeling solution, 2x conc.
   – Incubate the cells at +2 to +8°C for 30 minutes.

4. Wash 2 times with Incubation buffer.
   – Centrifuge the cells at 400 × *g* for 5 minutes after each washing step.

5. Analyze as outlined below.

Analysis

Flow cytometry (FACS)

Analyze the stained cells on a flow cytometer using 488 nm excitation and a 515 nm bandpass filter for fluorescein detection (Figure 1). Follow the instructions of the instrument manufacturer when using the instrument.

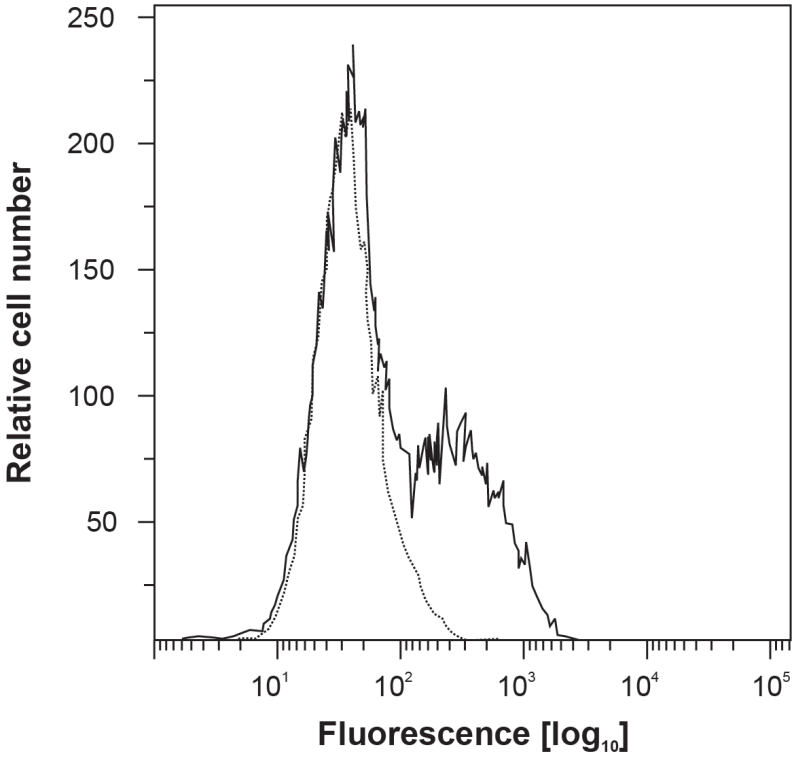

Fig. 1: Flow cytometry analysis of HA-tagged Burkitt Lymphoma Receptor 1 (BLR1-HA) detected with Anti-HA-Fluorescein, High Affinity (3F10).
HEK-293 cells were transfected with N-terminal HA-tagged BLR1. Cells were stained using Anti-HA-Fluorescein, High Affinity (3F10) as described above and analyzed by flow cytometry (FACS) analysis. Dotted lines represent mock-transfected control cells.

Confocal fluorescence microscopy

Evaluate the stained cells by fluorescence microscopy according to the instructions of the instrument manufacturer using 488 nm excitation and a 515 nm long pass filter for detection (Figure 2).

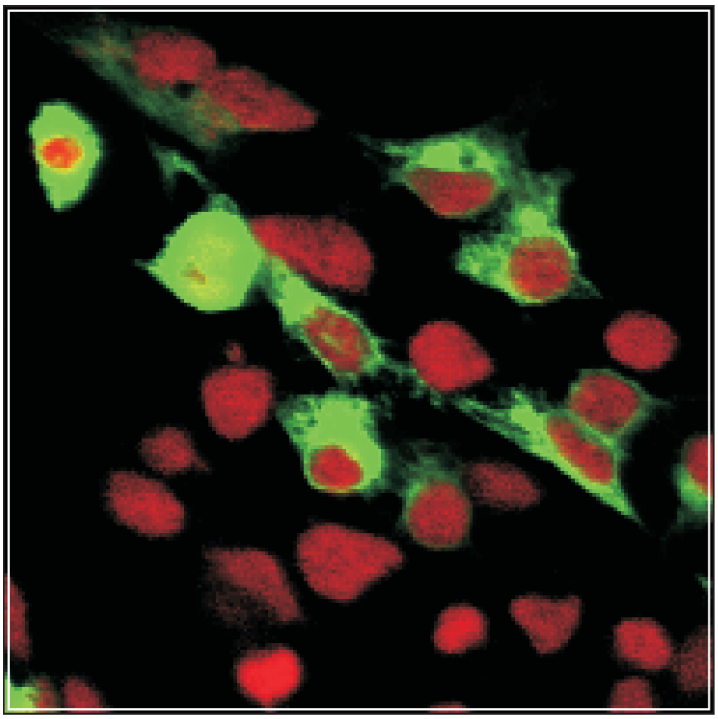

Fig. 2: Confocal fluorescence microscopy analysis of HA-tagged Burkitt Lymphoma Receptor 1 (BLR1-HA) detected with Anti-HA-Fluorescein, High Affinity (3F10).
HEK-293 cells were transfected with N-terminal HA-tagged BLR1. Cells were stained using Anti-HA-Fluorescein, High Affinity (3F10) as described above and analyzed by confocal fluorescence microscopy analysis. Red staining represents counterstaining of the nuclei using 7-Amino-actinomycin D (7-AAD).

2.3. Parameters

Specificity

Anti-HA-Fluorescein, High Affinity (3F10) recognizes the HA peptide sequence [YPYDVPDYA] derived from the human hemagglutinin protein. The antibody recognizes its antigenic determinant even when the HA peptide epitope is introduced into unrelated recombinant proteins by a technique known as epitope tagging.

Working Concentration

Use 1 to 5 µg/ml for flow cytometry and immunofluorescence.

3. Additional Information on this Product

3.1. Test Principle

Background information

The Anti-HA High Affinity antibody (clone 3F10) recognizes the same epitope as clone 12CA5, which was originally used to study how the immune system recognizes the influenza hemagglutinin protein, a surface glycoprotein required for infectivity of the human virus. However, the principal use of the Anti-HA antibody is the detection and purification of proteins whose encoding DNA sequences have been fused to the HA epitope sequence by recombinant techniques, that is epitope tagging. The ability to prepare such epitope-tagged proteins and locate them with the Anti-HA antibody in subsequent experiments has enabled researchers to determine:

- The size, cellular localization, and abundance of proteins produced by newly discovered genes.
- Post-translational modifications of proteins.
- The movement of proteins within cell membranes.
- The identity of proteins within functional protein complexes.
- The function of proteins that are unstable, difficult to purify, or share epitopes with a number of other proteins.

However, cross-reacting bands have been reported in certain western blot experiments using Anti-HA 12CA5. Anti-HA High Affinity is a monoclonal antibody whose high affinity and low working concentration result in less cross-reactivity when compared with other antibodies to the HA-epitope.

Preparation

1. Anti-HA High Affinity was obtained by immunizing mice with a synthetic peptide (residues 76-111 of X47 hemagglutinin 1) coupled to keyhole limpet hemocyanin (KLH).
2. Spleen cells were isolated and fused with P3-X63-Ag8.653 myeloma cells by standard methods.
3. Hybridoma supernatants were screened for specific binding to HA-epitope-tagged fusion proteins.
4. Hybridomas secreting monoclonal antibodies specific for the HA-epitope were isolated and cloned by limiting dilution.
5. The antibody was purified from bioreactor supernatants and lyophilized in the presence of proteinous stabilizers.

3.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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