OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to IgG3 - FITC

Catalog No.: AM05827FC-N

Quantity: 0.1 mg

Concentration: 0,1 mg/ml

Host: Human

Clone: 4582

Immunogen: Purified mouse monoclonal IgG3

Format: **State:** Liquid bivalent human recombinant Fab selected from the HuCAL® GOLD phage display library. Expressed in E. coli and purified using NiNTA affinity chromatography. This Fab fragment is dimerized via a helix-turn-helix motif. The antibody is tagged with a myc-tag (EQKLISEEDL) and a his-tag (HHHHHH) at the C-terminus of the antibody heavy chain.
Purification: Metal chelate affinity chromatography
Buffer System: Contains 0.09% Sodium Azide (NaN3) and 1% Bovine Serum Albumin
Label: FITC – Fluorescein isothiocyanate

Applications: ELISA: 1/100 - 1/200.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody is specific for IgG3. No cross reactivity is seen with other mouse immunoglobulin sub-classes, rat immunoglobulins, or sheep, bovine and goat IgG.
Species: Mouse.
Other species not tested.

Add. Information: **Hu**man **C**ombinatorial **A**ntibody **L**ibrary (HuCAL®) technology is a unique and innovative concept for in vitro generation of highly-specific and completely human antibodies - for a schematic drawing of available antibody formats see "Pictures" below.

Please Note:
HuCAL® antibodies are sold under license of U.S. Patents 6,300,064; 6,696,248; 6,708,484;
6,753,136; and European Patent 0,859,841 and corresponding patents.

Storage: Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols: Cited from:
HuCAL® Antibodies Technical Manual
First Edition
By Francisco Ylera

Use of HuCAL® antibodies in flow cytometry

HuCAL® antibodies are well suited for use in flow cytometry. Their small size and the absence of the Fc region usually results in low background and avoids potential interactions of the primary antibody with Fc receptors on cells. In addition, HuCAL® antibodies carry tags that allow them to be detected on antibody-expressing cells, such as B cells. The bivalent mini-antibody format (Fab-dHLX) is recommended, as it has higher avidity, but the monovalent format can also be used. For detection via the tag, we recommend a format with a FLAG® tag (e.g. Fab-dHLX-FH or Fab-FH), as the anti-FLAG® M2 secondary antibody has a higher affinity than the anti-His-6 tag antibody.

Protocols

(a) For HuCAL® antibodies and cells that do not produce human IgG

This protocol describes the use of HuCAL® antibodies in combination with an anti-human IgG conjugate that is specific for heavy and light chains, allowing detection of any Fab format, regardless of its tags. This method can be used to analyze antigen-positive cells that do not themselves display human IgG. Cells expressing the target antigen, and, where available, a negative control cell line, are required. All buffers used during the staining procedure must contain the appropriate supplements e.g. azide and ions, where necessary.

Recommended controls

- Background control: Use PBS instead of HuCAL® antibody, with FACS buffer and detection antibody.
- Negative control: Use unrelated negative control HuCAL® antibody, with FACS buffer and detection antibody.
- We recommend testing several dilutions of purified Fab for FACS and using a non-related Fab in identical dilutions as a negative control.
- Positive control (if available): Include with each FACS experiment to check whether the cells are expressing the selection antigen.

Preparation of FACS buffer

To prepare FACS buffer, mix ice-cold PBS, pH 7.4, 3% FCS, and 0.02% sodium azide. Centrifuge at high speed until the suspended matter is pelleted (approximately 30 minutes at 17,700 x g), at 4°C, and transfer supernatant to a new tube. Store at 4°C for up to one week.

Preparation of cells

1.

A. Adherent cells: Detach cells from their support using Trypsin/EDTA (Versene), and collect in a 50 ml sterile plastic tube.

B. Cells grown in suspension: Collect in a 50 ml sterile plastic tube.

2. Wash cells once in ice-cold FACS buffer and centrifuge at 190 x g for 5 minutes at 4°C. Wash cells by adding ice-cold FACS buffer to the tube, closing the tube, and inverting to mix.

3. Discard the supernatant, resuspend the cell pellet in the remaining drop, add 5 ml of ice-cold FACS buffer, and mix by inverting the tube.

4. Wash cells once in ice-cold FACS buffer and centrifuge at 190 x g for 5 minutes at 4°C.

5. Discard the supernatant, gently resuspend the cell pellet in the remaining drop, and add a small volume of ice-cold FACS buffer.

6. Stain cells using Trypan Blue and count the cells. Add FACS buffer to adjust the number of living cells to 0.5 x 10e6 cells/100 µl.

7. Add 100 µl of cell suspension (0.5 x 10e6 cells) to each well of a 96-well round bottom tissue culture plate, and centrifuge the plate at 716 x g for 2 minutes at 4°C.

8. Remove the supernatant and gently vortex the plate to bring the cells into suspension in the remaining liquid.

When using only a few wells, aspirate the supernatant with a pipette. If using an entire 96-well plate, invert the plate quickly but carefully over a tray to remove the supernatant.

Do not tap the plate on a paper towel, but place the plate on the bench and blot surface moisture from the top with a paper towel.

Procedure for cell staining

1. Prepare serial dilutions of HuCAL® antibodies, between 0.1 and 100 µg/ml in FACS buffer. 10 µg/ml is a standard concentration.
2. Add 100 µl of diluted antibody and controls to the wells containing antigen-expressing cells and the negative control cells. Incubate for 1 hour at 4°C with gentle agitation.
3. Centrifuge at 716 x g for 2 minutes at 4°C, remove the supernatant, and wash 2–3 times with 200 µl of FACS buffer.

When using only a few wells, aspirate the supernatant with a pipette. If using an entire 96-well plate, invert the plate quickly but carefully over a tray to remove the supernatant. Do not tap the plate on a paper towel, but place the plate on the bench and blot surface moisture from the top with a paper towel.

4. Add 100 µl of R-Phycoerythrin-conjugated goat anti-human IgG (H+L) specific F(ab')2 fragment secondary antibody to the wells (samples and controls) containing antigen-expressing cells and the negative control cells. Incubate for 1 hour at 4°C with gentle agitation.
5. Centrifuge at 716 x g for 2 minutes at 4°C, remove the supernatant, and wash 2–3 times with 200 µl of FACS buffer.

When using only a few wells, aspirate the supernatant with a pipette. If using an entire 96-well plate, invert the plate quickly but carefully over a tray to remove the supernatant. Do not tap the plate on a paper towel, but place the plate on the bench and blot surface moisture from the top with a paper towel.

6. After the last washing step, centrifuge again for 2 minutes at 4°C, resuspend cells in 100–200 µl of FACS buffer, and transfer to FACS tubes. For short-term storage (up to 4 days), stained cells can be resuspended in 100 µl of 4% paraformaldehyde in PBS after the final washing step. Cover cells with aluminium foil and store at 4°C. Immediately before FACS analysis, add FACS buffer to give the required volume.

(b) For cells that produce human IgG

This protocol describes the detection of HuCAL® antibodies with anti-tag antibodies. It is well suited for staining cells that produce and expose human IgG on their surface and, therefore, cannot be analyzed using an anti-human IgG specific antibody. Depending upon the tags on the HuCAL® antibodies, different detection antibodies can be used (anti-FLAG® M2 antibody, anti-c-Myc antibody, or anti-His-6 antibody), followed by a conjugated anti-mouse IgG antibody. Cells expressing the target antigen, and, where available, a negative control cell line, are required. All buffers used during the staining procedure must be supplemented with azide, appropriate ions etc.

Recommended controls

- Two background controls: Use PBS instead of HuCAL® antibody, with FACS buffer, with and without an anti-tag antibody and detection antibody.
- Negative control: Use unrelated negative control HuCAL® antibody, with FACS buffer and detection antibody.
- Positive control (if available): Include a positive control with each FACS experiment wherever possible, to check whether the cells are expressing the selected antigen.
- We recommend testing several dilutions of purified Fab for FACS and using a non-related Fab in identical dilutions as negative control.

Preparation of cells

1. Follow the protocol described before.

Procedure for cell staining

1. Prepare serial dilutions of HuCAL® antibodies, between 0.1-100 µg/ml in FACS buffer. 10 µg/ml is a standard concentration.
2. Add 100 µl of diluted antibody and controls to the wells containing antigen-expressing cells and the negative control cells. Incubate for 1 hour at 4°C with gentle agitation.

3. Centrifuge at 716 x g for 2 minutes at 4°C, remove the supernatant, and wash 2–3 times with 200 µl of FACS buffer.
When using only a few wells, aspirate the supernatant with a pipette. If using an entire 96-well plate, invert the plate quickly but carefully over a tray to remove the supernatant. Do not tap the plate on a paper towel, but place the plate on the bench and blot surface moisture from the top with a paper towel.
4. Add the secondary antibody.
A. For HuCAL® antibodies with a FLAG® tag (e.g. Fab-dHLX-FH or Fab-FH), add 100 µl of anti-FLAG® M2 antibody (Sigma, Cat. No. F3165) to each well, diluted 1:2000 in FACS buffer.
B. For HuCAL® antibodies with a c-Myc tag (e.g. Fab-dHLX-MH or Fab-MH), add 100 µl of anti-c-Myc antibody to each well, diluted 1:2000 in FACS buffer.
C. For HuCAL® antibodies with a His-6 tag (e.g. Fab-dHLX-MH or Fab-MH), add 100 µl of anti-His antibody to each well (e.g. Acris Antibodies cat.no. SM1693P) diluted 1:500 in FACS buffer.
Anti-c-Myc antibody can give higher background with endogenous c-Myc of dead cells. We therefore recommend the use of the anti-FLAG® M2 antibody where possible.
5. Add FACS buffer to one background control well, and anti-tag antibody to the second background control well.
6. Incubate the plates for 1 hour at 4°C, with gentle agitation on an ELISA shaker.
7. Centrifuge at 716 x g for 2 minutes at 4°C, remove the supernatant, and wash 2–3 times with 200 µl of FACS buffer.
When using only a few wells, aspirate the supernatant with a pipette. If using an entire 96-well plate, invert the plate quickly but carefully over a tray to remove the supernatant. Do not tap the plate on a paper towel, but place the plate on the bench and blot surface moisture from the top with a paper towel.
8. Add the tertiary antibody: 100 µl of R-Phycoerythrin-conjugated goat anti-mouse IgG antibody to each well, diluted 1:500 in FACS buffer.
9. Incubate the plates for 1 hour at 4°C, with gentle agitation on an ELISA shaker.
10. Centrifuge for 2 minutes at 4°C, remove the supernatant, and wash 2–3 times with 200 µl of FACS buffer. When using only a few wells, aspirate the supernatant with a pipette. If using an entire 96-well plate, invert the plate quickly but carefully over a tray to remove the supernatant. Do not tap the plate on a paper towel, but place the plate on the bench and blot surface moisture from the top with a paper towel.
11. After the last washing step, centrifuge again for 2 minutes at 4°C, resuspend cells in 100–200 µl of FACS buffer, and transfer to FACS tubes. For short-term storage (up to 4 days), stained cells can be resuspended in 100 µl of 4% paraformaldehyde in PBS after the final washing step. Cover cells with aluminium foil and store at 4°C. Immediately before FACS analysis, add FACS flow buffer to give the required volume.

Pictures:

Schematic drawing of the main antibody formats that are available: Monovalent Fab with two tags (left); Bivalent Fab with two tags, functionally equivalent to a F(ab')2 fragment (middle); Full IgG (right). Monovalent and bivalent Fab antibodies are purified after bacterial expression via an epitope tag (red - orange).