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Monoclonal Antibody to IgG2a - Purified

Catalog No.: AM05825PU-S

Quantity: 50 µg

Concentration: 1,0 mg/ml (after reconstitution)

Host: Human

Clone: 6344

Immunogen: Purified mouse monoclonal IgG2a

Format:
State: Lyophilised 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
Reconstitution: Reconstitute with 0.05ml distilled water

Applications:
ELISA: 1/1000 - 1/2500.
Flow Cytometry: 1/10.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
This antibody is specific for mouse IgG2a. 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:
Recommended Secondary Antibodies:
Mouse Anti Human CMYC (SM1863P) HRP
Mouse Anti Synthetic Peptide HISTIDINE TAG (SM1693P) HRP

Human **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:
Prior to reconstitution store at 2-8°C.
Following reconstitution store the antibody at -20°C.
Avoid repeated freezing and thawing.
Shelf life: 6 month from despatch.

Protocols:

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

Use of HuCAL® antibodies in ELISA
ELISA can be performed using HuCAL® antibodies in protocols used for other poly- or monoclonal antibodies, provided a suitable secondary antibody is used. As HuCAL® antibodies do not contain the Fc domain, we recommend using an anti-human Fab secondary antibody (use of polyclonal antiserum amplifies the signal). Monoclonal antibodies against the tag (e.g. anti-Myc, anti-His-6) can also be used. The bivalent format of the HuCAL® mini-antibodies (Fab-dHLX or Fab-A) is recommended for ELISA assays with immobilized antigen because their avidity is higher, similar to that of full IgGs.
(A) Indirect ELISA
Indirect ELISA is recommended as a control assay to test the performance of reagents. In indirect ELISA, the antigen is immobilized on a surface, such as a well of a microtiter plate. The plate is blocked to prevent non-specific binding of antibodies, and a specific antibody is added to each well. The plate is washed to remove unbound antibody, leaving only the specific antigen-antibody complexes of interest. A secondary antibody is added. This is conjugated to an enzyme such as horseradish peroxidase (HRP) or alkaline phosphatase, and binds the antibody-antigen complexes. The plate is washed and the appropriate substrate is applied. The resulting chromogenic or fluorescent signal is viewed using a spectrophotometer.
Protocol
Indirect ELISA with fluorescence readout
1. Coat the antigen by adding 20 µl of a solution of 5 µg/ml antigen in PBS to several wells of a 384-well Maxisorp(TM) microtiter plate, and incubate overnight at 4°C.
Use a black, 384-well ELISA microtiter plate: square well, flat bottom MaxiSorp(TM) PS (NUNC, Cat. No. 460518).
2. Wash the microtiter plate twice with PBST.
3. Block the microtiter plate by adding 100 µl of 5% non-fat dried milk in PBST to each well, and incubate for 1–2 hours at room temperature.
4. Wash the microtiter plate twice with PBST.
5. Transfer 20 µl of HuCAL® antibody to each well. We recommend a standard concentration of 2 µg/ml in PBST or HiSpec buffer BUF049). Incubate for 1 hour at room temperature.
Titrate using different concentrations of HuCAL® antibody.
6. Wash the microtiter plate 5 times with PBST.
7. Add 20 µl of secondary antibody to each well and incubate for 1 hour at room temperature.
We recommend using anti-human Fab:AP .
8. Wash the microtiter plate 5 times with PBST.
9. Add 20 µl of AttoPhos® (Roche) to each well and measure fluorescence after 10 minutes (excitation: 440±25 nm, emission: 550±35 nm).
Note: Instead of the 384-well format, a 96-well plate can be used (e.g. black, flat bottom MaxiSorp(TM)PS, NUNC, Cat. No. 437111). For the 96-well format use 100 µl instead of 20 µl of antigen, antibodies or substrate and 300 µl for the blocking step. Instead of an alkaline phosphatase-conjugated secondary antibody, an
HRP-conjugated anti-Fab can be used in combination with QuantaBlu® reagent (Pierce Cat. No. 15169, excitation: 320±25 nm, emission: 430±35 nm).
If detection is done with a chromagenic substrate (e.g. BM Blue Soluble Peroxide Substrate, Roche, Cat. No. 1484281), transparent plates must be used.
(B) Direct ELISA

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Material Safety Datasheets are available at www.acris-antibodies.com or on request.
Acris Antibodies is now part of the OriGene family. Learn more at www.origene.com

In direct ELISA, the primary antibody is labeled, for example, by genetic fusion with alkaline phosphatase. This avoids use of a secondary antibody, saving time and money, which is valuable for assays that are frequently run. However, the system lacks the amplification effect generated by the secondary antibody in indirect ELISA and is therefore less sensitive.

Protocol

Direct ELISA with alkaline phosphatase-conjugated primary antibody and fluorescence readout

1. Coat the antigen by adding 20 µl of a solution of 5 µg/ml antigen in PBS to several wells of a 384-well Maxisorp(TM) microtiter plate, and incubate overnight at 4°C.
Use a black, 384-well ELISA microtiter plate: square well, flat bottom, MaxiSorp(TM) PS (NUNC, Cat. No. 460518).
2. Wash the microtiter plate twice with PBST.
3. Block the microtiter plate by adding 100 µl of 5% non-fat dried milk in PBST to each well, and incubate for 1-2 hours at room temperature.
4. Wash the microtiter plate twice with PBST.
5. Transfer 20 µl of labeled HuCAL® antibody to each well. We recommend a standard concentration of 2 µg/ml in PBST. Incubate for 1 hour at room temperature. Titrate using different concentrations of HuCAL® antibody.
6. Wash the microtiter plate 5 times with PBST.
7. Add 20 µl of AttoPhos® (Roche) to each well and measure the fluorescence after 10 minutes (excitation: 440±25 nm, emission: 550±35 nm).

(C) Sandwich ELISA

Sandwich ELISA is a very sensitive and specific method of detecting antigens, and provides fast and accurate determination of the concentration of antigen in a sample. If a purified antigen standard is available, the method can also measure the absolute concentration of antigen in a given sample. The technique uses two antibodies, both of which are specific to the antigen of interest, and which bind the antigen at non-overlapping epitopes. In sandwich ELISA, it is possible to use a HuCAL® antibody as the capture or detection antibody together with an existing commercial antibody, or to use two different HuCAL® antibodies as the sandwich pair. The bivalent format of HuCAL® mini-antibodies is highly suitable for both capture and detection. When two HuCAL® antibodies are used, detection is best performed using a labeled anti-tag tertiary antibody (e.g. Fab-A-FH HuCAL® antibody with HRP-conjugated rabbit anti-bacterial alkaline phosphatase). Alternatively, the detection antibody can be biotinylated and visualized with streptavidin-AP.

Protocols

Sandwich ELISA using two HuCAL® antibodies

1. Coat the capture antibody by adding 20 µl of a solution of 5 µg/ml in PBS to several wells of a 384-well Maxisorp(TM) microtiter plate, and incubate overnight at 4°C.
Use a black, 384-well ELISA microtiter plate: square well, flat bottom, MaxiSorp(TM) PS (NUNC, Cat. No. 460518).
2. Wash the microtiter plate twice with PBST.
3. Block the microtiter plate by adding 100 µl of 5% BSA in PBST to each
well, and incubate for 1 hour at room temperature.
4. Wash the microtiter plate twice with PBST.
5. Add 20 µl of antigen to each well of the microtiter plate, and incubate for 1 hour at room temperature.
Use a concentration range of the antigen in PBS.
6. Wash the microtiter plate 5 times with PBST.
7. Add 20 µl of HuCAL® detection antibody in Fab-A-FH format (2 µg/ml concentration in PBST or HiSpec buffer) to each well, and incubate for 1 hour at room temperature.
8. Wash the microtiter plate 5 times with PBST.
9. Transfer 20 µl of HRP-conjugated rabbit anti-bacterial alkaline phosphatase antibody

and incubate for 1 hour at room temperature.
Use rabbit anti-bacterial alkaline phosphatase.
10. Wash the microtiter plate 5 times with PBST.
Add 20 µl of QuantaBlu® (Pierce) to each well and measure the fluorescence directly (excitation: 320±25 nm, emission: 420±35 nm).
Sandwich ELISA with polyclonal mouse and HuCAL® antibody
1. Coat the HuCAL® capture antibody by adding 20 µl of a solution of 5 µg/ml in PBS to several wells of a 384-well Maxisorp(TM) microtiter plate, and incubate overnight at 4°C.
2. Wash the microtiter plate twice with PBST.
3. Block the microtiter plate by adding 100 µl of 5% BSA in PBST to each well, and incubate for 1 hour at room temperature.
4. Wash the microtiter plate twice with PBST.
5. Add 20 µl of antigen to each well of the microtiter plate, and incubate for 1 hour at room temperature.
Use a concentration range of the antigen in PBS .
6. Wash the microtiter plate 5 times with PBST.
7. Add 20 µl of biotinylated polyclonal detection antibody (concentration as specified by the manufacturer) in HiSpec buffer to each well, and incubate for 1 hour at room temperature.
8. Wash the microtiter plate 5 times with PBST.
9.Transfer 20 µl alkaline phosphatase conjugated streptavidin and incubate for 1 hour at room temperature.

(D) Competition (or inhibition) ELISA

Competition (or inhibition) ELISA is ideal when only one suitable antibody is available for the target of interest or when the antigen is too small to be detected e.g. a hapten. The technique measures the concentration of a substance by its ability to interfere with an established pre-titrated system. The primary antibody is first incubated with the free antigen. It is then added to an antigen-coated well, and the plate is washed to remove unbound antibody. The amount of antibody that binds the immobilized antigen is detected using a secondary antibody linked to a detection system such as horseradish peroxidase. The appropriate substrate is applied and the resulting chromogenic or fluorescent signal is viewed using a spectrophotometer. The higher the concentration of free antigen in the sample, the less antibody is available to bind the immobilized antigen, and, therefore, the weaker the signal; conversely, lower amounts of free antigen in solution generate stronger signals. Other variations of competition ELISA are of course possible.

Protocol

1. Coat the antigen by adding 50 µl of a solution of 5 µg/ml antigen in PBS to several wells of a 384-well Maxisorp(TM) microtiter plate, and incubate overnight at 4°C.
2. Wash the microtiter plate twice with PBST.
3. Block the microtiter plate by adding 100 µl of 5% non-fat dried milk in PBST to each well, and incubate for 1-2 hours at room temperature.
4. Incubate 25 µl of antigen solution with 25 µl of HuCAL® antibody (final concentration 2 µg/ml) for 1 hour at room temperature. Use a range of concentrations of the antigen in solution, in PBS
5. Wash the microtiter plate twice with PBST.
6. Transfer 50 µl of the HuCAL® antibody/antigen mix to each well of the microtiter plate, and incubate for 1 hour at room temperature.
7. Wash the microtiter plate 5 times with PBST.
8. Transfer 50 µl of secondary antibody (anti-human Fab:AP and incubate for 1 hour at room temperature.
9. Wash the microtiter plate 5 times with PBST.
10. Add 20 µl of AttoPhos® (Roche) to each well, incubate for 10 minutes at room temperature, and measure fluorescence (excitation: 440±25nm, emission: 550±35nm).

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).