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Monoclonal Antibody to CRIT - Purified

Catalog No.: AM01298PU-N

Quantity: 0.1 mg

Concentration: 0.5 mg/ml

Background: CRIT binds complement factor C2 and blocks the classical pathway of complement activation. CRIT protein expression has been specifcally identified by immunohistochemistry in podocyte cells in human kidney glomeruli, and is also expressed on hematopoietic cells and a range of other human tissues. CRIT appears to function as a regulatory molecule protecting autologous cells from damage by complement. Expression has also been demonstrated by human parasites, which have acquired CRIT by horizontal transmission.

Uniprot ID: Q5I7P2

NCBI: 9606

Host / Isotype: Human / Ig

Clone: 4644

Immunogen: Synthetic peptide derived from the first 27 amino acids of the first extracellular domain of human CRIT conjugated to BSA and Transferrin.
AA Sequence:
MSPSLVSYTQKNERGSHEVKIKHFSPY

Format: **State:** A lyophilised bivalent human recombinant Fab selected from the HuCAL® GOLD phage display library. Expressed in E. coli and purified using NiNTA affinity chromatography (Metal-chelate 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 anti-body heavy chain.
Reconstitution: Restore with 0.1 ml distilled water.

Applications: ELISA.
Western Blot: 1/100 - 1/500.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody recognises the novel complement regulatory cell surface receptor designated CRIT (Complement C2 receptor inhibitor trispanning).
Species: Human.
Other species not tested.

Add. Information: **Recommended Secondary Antibodies:**
MOUSE ANTI HISTIDINE TAG:HRP (SM1693HRP)
MOUSE ANTI C-MYC:HRP (SM1863HRP)
GOAT ANTI HUMAN IgG F(ab')2:HRP (AP05000HR-N)

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 at -20°C.
Avoid repeated freezing and thawing.
Shelf life: 6 months from despatch.

General Readings:

1. Inal JM, Hui KM, Miot S, Lange S, Ramirez MI, Schneider B, et al. Complement C2 receptor inhibitor trispanning: a novel human complement inhibitory receptor. J Immunol. 2005 Jan 1;174(1):356-66. PubMed PMID: 15611259.

Protocols:

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

Use of HuCAL® antibodies in Western blotting
HuCAL® antibodies can be used for Western blotting following the standard protocols developed for conventional antibodies. The only difference is that the secondary antibody cannot be directed against the Fc domain, as this is lacking in HuCAL® Fab antibodies. Therefore, we suggest using an anti-human Fab secondary antibody due to the signal amplification with polyclonal secondaries or an antibody against the tag, for instance, anti-Myc. The sensitivity of an antibody in a given application depends on its affinity and avidity. Affinity is the strength with which the antibody binds its antigen in a 1:1 interaction. Avidity is the strength with which an antibody with multiple binding sites binds its antigens. Antibodies with multiple binding sites have better binding properties than monovalent antibodies when multiple epitopes are presented on a solid support. The affinities of HuCAL® antibodies are comparable to those of other monoclonal antibodies. Bivalent antibodies have higher sensitivity than monovalent antibodies as their avidity is greater. The bivalent format of HuCAL® antibodies, is therefore, recommended for most applications, including Western blotting.
Protocol
1. Run the sample on SDS-PAGE and transfer onto PVDF membrane. For initial testing, use 200 µg of cell lysate proteins or 300 ng of pure antigen.
2. Block the membrane with 5% non-fat dried milk in TBST for 1 hour on a shaker or overnight at 4°C.
3. Rinse the membrane with TBST.
4. Add HuCAL® antibody (primary antibody) to the membrane in TBST with 1% non-fat dried milk.
The optimal amount of HuCAL® antibody is typically between 1 and 10 µg/ml, but must be determined for each antibody. We recommend starting with a concentration of 5 µg/ml.
5. Incubate for 1 hour at room temperature on a shaker.
Ensure that the membrane is completely immersed in the buffer.
6. Wash the membrane 3 times, each for 5 minutes with TBST.
Use a generous amount of TBST.
7. Add secondary antibody to the membrane.
We recommend using anti-human Fab:HRP
8. Shake for 1 hour at room temperature.
9. Wash the membrane 3 times, each for 5 minutes with TBST.
Use a generous amount of TBST.
10. Develop the membrane using ECL Plus(TM) or ECL Advance(TM) (GE Healthcare) according to the manufacturer's instructions, and use an imager or X-ray film for detection.

Note: We strongly recommend sonication of lysates with ultrasound to disrupt genomic DNA in order to reduce non-specific background signals. Depending on the format of the HuCAL® Fab, an anti-tag secondary antibody can be used.

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

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

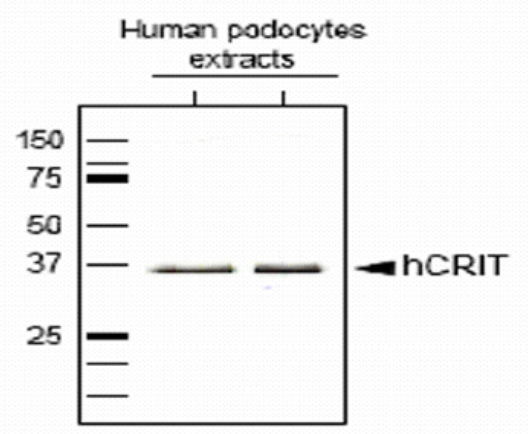
Competition (or inhibition) ELISA is ideal when only one suitable antibody is available for the target of interest or when the antigen is too small to be detected e.g. a hapten. The technique measures the concentration of a substance by its ability to interfere with an established pre-titrated system. 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).

Pictures:

Western blot detection of human CRIT in podocyte cell extract. Note visualisation of target protein at approximately 36kD. AM01298PU-N was detected using Goat anti-human IgG (Fab')2 (AP05000HR-N).



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).