

Reagents Provided

Alexa Fluor® 488-conjugated mouse monoclonal anti-rat CD44: Supplied as 10 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 740017

Isotype: mouse IgG_{2b}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

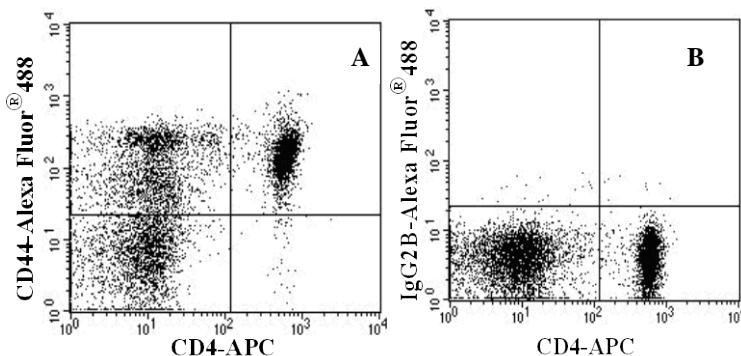
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD44 within a population and qualitatively determine the density of CD44 on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified NS0 derived recombinant rat CD44 (rhCD44; Accession # P26051) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of CD44 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515-545 nm.



Rat splenocytes were stained with APC-conjugated anti-rat CD4 and either A) Alexa Fluor® 488-conjugated anti-rat CD44 (Catalog # FAB6577G) or B) Alexa Fluor® 488-conjugated isotype control (Catalog # IC0041G).

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides.

Flush with large volumes of water during disposal.

Legal

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Background Information

CD44 is a ubiquitously expressed protein that is the major receptor for hyaluronan and exerts control over cell growth and migration.¹⁻⁵ The many reported functions of CD44 fall within three categories.^{1,2} First, CD44 binds hyaluronan and other ligands within the extracellular matrix and can function as a "platform" for growth factors and metalloproteinases. Second, CD44 acts as a co-receptor, which can modify the activity of receptors including MET and the ErbB family of tyrosine kinases. Third, the CD44 intracellular domain links the plasma membrane to the actin cytoskeleton via the ERM proteins, ezrin, radixin and moesin. CD44 can be synthesized in a soluble form or may be cleaved at multiple sites by either membrane-type matrix metalloproteinases, or ADAM proteases to produce soluble ectodomains.^{4,6,7} The cellular portion may then undergo gamma secretase-dependent intramembrane cleavage to form an Aβ-like transmembrane portion and a cytoplasmic signaling portion that affects gene expression.^{8,9} These cleavage events are thought to promote metastasis by enhancing tumor cell motility and growth.^{1,2,6} Within the N-terminal invariant portion of the ECD (aa 23-222), mouse CD44 shares 92%, 77%, 77%, 79%, and 71% amino acid sequence identity with rat, human, equine, canine, and bovine CD44, respectively.

References

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Flow Cytometry Validation

This antibody has been tested for flow cytometry using rat splenocytes.

- Cells may be Fc-blocked with 1 µg of rat IgG/10⁵ cells for 15 minutes at room temperature. Do not wash away excess blocking IgG.
- After blocking, 5 µL of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 488-labeled mouse IgG_{2b} antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.