

Technical Data Sheet

Purified Mouse anti-CRABP2

Product Information

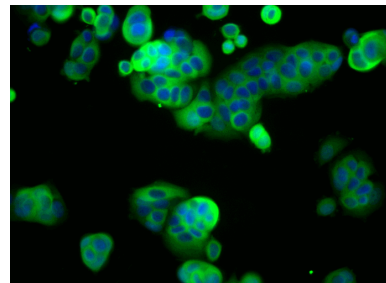
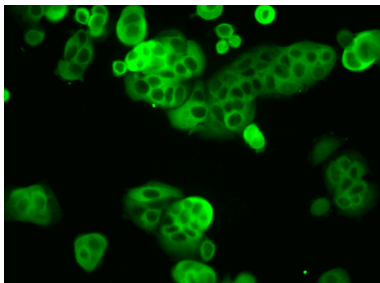
Material Number:	560234
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	M60-1228
Immunogen:	Human CRABP2 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	Tested: Human Predicted: Mouse
Target MW:	15 kDa
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

Description

The M60-1228 monoclonal antibody reacts with Cellular Retinoic Acid-Binding Protein 2 (CRABP2). CRABP2 is a cytosolic low-molecular-weight protein with high affinity for retinoic acid (RA), a member of the vitamin A family that is able to induce cell differentiation. The translocation of CRABP2 to the nucleus indicates that it may function to deliver RA to RA receptors, which activate gene transcription. The differential expression of the *CRABP2* gene during development and its overexpression in many cancers suggest that it is important in RA-mediated regulation of cell growth and differentiation.



Western blot analysis of CRABP2 in human breast adenocarcinoma. MCF7 Cell Lysate (Cat. No. 611548) was probed with Purified Mouse anti-CRABP2 monoclonal antibody at titrations of 0.25 (lane 1), 0.05 (lane 2), and 0.01 $\mu\text{g/ml}$ (lane 3). CRABP2 is identified as a band of 15 kDa.



Immunofluorescent staining of human breast adenocarcinoma. MCF7 cells (ATCC HTB-22) were seeded in a 96-well imaging plate (Cat. No. 353219) at $\sim 10,000$ cells per well. After overnight incubation, the cells were fixed, permeabilized with Triton™ X-100, and stained with Purified Mouse anti-CRABP2 (pseudo colored green) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 647 goat anti-mouse Ig (Invitrogen), and counterstaining was with Hoechst 33342 (pseudo colored blue). The left image shows CRABP2 alone, and the right image shows CRABP2 merged with Hoechst staining. Images were captured on a BD Pathway™ 435 Cell Analyzer using a 20x objective and merged using BD AttoVision™ software. This antibody also worked with the Saponin fix/perme protocol (see Recommended Assay Procedure).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4° C.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

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Recommended Assay Procedure

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytotfix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 µl of 1× PBS.
5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c):
 - a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
 - b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
 - c. Add 100 µl of 1× Perm/Wash buffer (Cat. No. 554723) to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.
6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 µl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).
7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.
8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.
9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.
10. Remove the antibody, and wash the wells three times with 100 µl of wash buffer. An optional detergent wash (100 µl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.
11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.
12. After the final wash, counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
2. Triton is a trademark of the Dow Chemical Company.

References

Gupta A, Williams BRG, Hanash SM, Rawwas J. Cellular retinoic acid-binding protein II is a direct transcriptional target of MycN in neuroblastoma. *Cancer Res.* 2006; 66(16):8100-8108.(Biology)
Lane MA, Xu J, Wilen EW, Sylvester R, Derguini F, Gudas LJ. LIF removal increases CRABP I and CRABP II transcripts in embryonic stem cells cultured in retinol or 4-oxoretinol. *Mol Cell Biol.* 2008; 28(1-2):63-74.(Biology)