Technical Data Sheet

# Purified Mouse Anti-Human Recoverin

## Product Information

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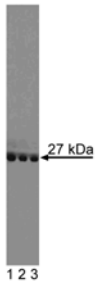
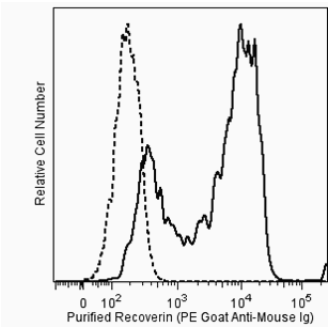
|---|---|
| **Material Number:** | **564905** |
| **Alternate Name:** | Protein CAR; cancer-associated retinopathy protein; RCVRN; RCV1 |
| **Size:** | 0.1 mg |
| **Concentration:** | 0.5 mg/ml |
| **Clone:** | S36-208 |
| **Immunogen:** | Human Recoverin Recombinant Protein |
| **Isotype:** | Mouse IgG1, κ |
| **Reactivity:** | QC Testing: Human<br>Lack of Reactivity Confirmed in Development: Mouse, Rat |
| **Target MW:** | 27 kDa |
| **Storage Buffer:** | Aqueous buffered solution containing ≤0.09% sodium azide. |

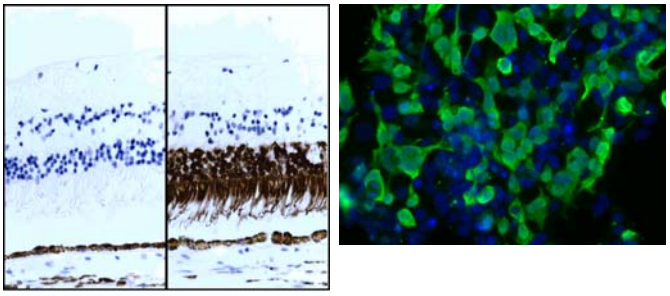
## Description

The S36-208 monoclonal antibody specifically binds to recoverin, a member of the recoverin family of neuronal calcium sensors. Recoverin contains an EF-hand type Ca2+-binding site and is expressed in vertebrate photoreceptors, certain retinal neurons, and pineal glands. Recoverin plays a role in the recovery phase of visual excitation and in adaptation to background light. Recoverin is used as a marker to evaluate the differentiation of pluripotent stem cells into rod and cone photoreceptors or cone bipolar cells. Recoverin is also a paraneoplastic antigen classified as a cancer-retina antigen. Although normally expressed only in the nervous system, aberrant expression of recoverin is detected in tumors of the lung and other tissues.




***Western blot and flow cytometric analyses of Recoverin expression.***
*LEFT: A lysate of human Recoverin-transfected 293-F cells was was prepared, electrophoresed (SDS-PAGE) and transferred to a PVDF membrane. The membrane was probed with serial two-fold dilutions of Purified Mouse Anti-Human Recoverin. Lanes 1, 2, and 3 show 62.5 ng, 31.25 ng, and 15.62 ng per lane, respectively. Recoverin is identified as a band of ~27 kDa. Specific staining was detected with HRP Goat Anti-Mouse Ig (Cat No 554002). The Purified Mouse Anti-Human Recoverin was similarly tested on mouse and rat eye lysates and was found to be negative (data not shown).*
*RIGHT: Human Recoverin-transfected 293-F cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), frozen, thawed, and then permeabilized with BD Phosflow™ Perm/Wash Buffer I (Cat. No. 557885). The permeabilized cells were stained with either Purified Mouse Anti-Human Recoverin (solid-line histogram) or Purified Mouse IgG1 κ Isotype Control (Cat. No. 554121, dashed-line histogram), followed by the second-step reagent PE Goat Anti-Mouse Ig (Cat. No. 550589), washed 1x with Perm/Wash Buffer I, then resuspended in BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656). The overlayed histograms were derived from gated events with forward and side light-scatter characteristics of intact cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System. Permeabilization with BD Pharmingen™ Transcription Factor Buffer Set (Cat No. 562574 or 562725) is also suitable for use with this antibody.*



***Immunohistochemical and immunofluorescent analyses of Recoverin expression.***
*LEFT: Following antigen retrieval with BD Retrievagen A Buffer (Cat. No. 550524), the formalin-fixed paraffin-embedded sections of human retina were stained with either Purified Mouse IgG1 κ Isotype Control (Cat. No.550878; Left Image) or Purified Mouse Anti-Human Recoverin (Right Image). A three-step staining procedure that employs Biotin Goat Anti-Mouse Ig (Cat. No. 550337), Streptavidin HRP (Cat. No. 550946), and the DAB Substrate Kit (Cat. No. 550880) was used to develop the primary staining reagents. As shown in the right image, the recoverin-specific antibody primarily stained rod and cone cells. Original magnification: 40×.*
*RIGHT: Recoverin-transfected 293-F cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655), permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050), and stained with Purified Mouse Anti-Human Recoverin (pseudo-colored green) at 1.2µg/mL. The second-step reagent was Alexa Fluor® 488 goat anti-mouse Ig ( Life Technologies), and the nuclear counter-staining was with DAPI (pseudo-colored blue). The images were captured on a BD Pathway™ 435 Cell Analyzer and merged using BD Attovision™ software. BD Perm/Wash™ Buffer (Cat No. 554723) is also suitable for use with this antibody.*

## Preparation and Storage

Store undiluted at 4°C.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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## Application Notes

**Application**

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|---|---|
| Intracellular staining (flow cytometry) | Routinely Tested |
| Western blot | Tested During Development |
| Bioimaging | Tested During Development |
| Immunofluorescence | Tested During Development |
| Immunohistochemistry-formalin (antigen retrieval required) | Tested During Development |

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 554655 | Fixation Buffer | 100 mL | (none) |
| 557885 | Perm/Wash Buffer I | 125 mL | (none) |
| 554121 | Purified Mouse IgG1 κ Isotype Control | 0.1 mg | MOPC-21 |
| 554656 | Stain Buffer (FBS) | 500 mL | (none) |
| 550524 | Retrievagen A (pH 6.0) | 1000 mL | (none) |
| 550878 | Purified Mouse IgG1 κ Isotype Control | 1 mL | MOPC-31C |
| 550337 | Biotin Goat Anti-Mouse Ig (Multiple Adsorption) | 1 mL | Polyclonal |
| 550946 | Streptavidin HRP | 50 mL | (none) |
| 550880 | DAB Substrate Kit | 500 Tests | (none) |
| 558050 | Perm Buffer III | 125 mL | (none) |
| 554723 | Perm/Wash Buffer | 100 mL | (none) |
| 550589 | PE Goat Anti-Mouse Ig (Multiple Adsorption) | 0.2 mg | Polyclonal |
| 554002 | HRP Goat Anti-Mouse Ig | 1 mL | (none) |
| 562574 | Transcription Factor Buffer Set | 100 Tests | (none) |
| 562725 | Transcription Factor Buffer Set | 25 Tests | (none) |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
5. 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.

## References


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Lamba DA, McUsic A, Hirata RK, Wang PR, Russell D, Reh TA. Generation, purification and transplantation of photoreceptors derived from human induced pluripotent stem cells. *PLoS ONE*. 2010; 5(1). (Biology)

Maeda A, Ohguro H, Maeda T, et al. Aberrant expression of photoreceptor-specific calcium-binding protein (recoverin) in cancer cell lines. *Cancer Res.* 2000; 60(7):1914-1920. (Biology)

Mellough CB, Sernagor E, Moreno-Gimeno I, Steel DH, Lako M. Efficient stage-specific differentiation of human pluripotent stem cells toward retinal photoreceptc cells. *Stem Cells*. 2012; 30(4):673-686. (Biology)

Meyer JS, Howden SE, Wallace KA, et al. Optic vesicle-like structures derived from human pluripotent stem cells facilitate a customized approach to retinal disease treatment. *Stem Cells*. 2011; 29(8):1206-1218. (Biology)

Murakami A, Yajima T, Inana G. Isolation of human retinal genes: recoverin cDNA and gene. *Biochem Biophys Res Commun.* 1992; 187(1):234-244. (Biology)



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