



AssayMax Human Recoverin ELISA Kit

Catalog # ER5010-1

Introduction

Recoverin is a calcium-dependent inhibitor of rhodopsin kinase, a member of the EF-hand family of calcium-binding proteins involved in the transduction of light by vertebrate photoreceptors. Recoverin also was identified as an autoantigen in the degenerative disease of the retina known as cancer-associated retinopathy (CAR), a paraneoplastic syndrome whereby immunological events lead to the degeneration of photoreceptors in some individuals with cancer (1 - 3).

Principal of the Assay

The AssayMax Human Recoverin ELISA kit is designed for detection of human recoverin in plasma, serum, tissue and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures recoverin in 3.5 hours. A polyclonal antibody specific for recoverin has been pre-coated onto a microplate. Recoverin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for recoverin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Recoverin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human recoverin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Recoverin Standard:** Recombinant human recoverin in a buffered protein base (40 ng, lyophilized).
- **Biotinylated Recoverin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against recoverin (80 μ l).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 μ l).
- **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x):** A 10-fold concentrated buffered surfactant (2 x 30 ml).

- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2 - 8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8⁰C. Store reconstituted standard at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Use undiluted samples and assay immediately. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 min. Collect the supernatant and measure the protein concentration. Use undiluted samples or 1:10 diluted samples with EIA Diluent and assay. The undiluted samples can be stored at -20⁰C or below.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Recoverin Standard:** Reconstitute the 40 ng of human recoverin standard with 2 ml of EIA Diluent to generate a stock solution of 20 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (20 ng/ml) 1:4 with EIA Diluent to produce 5, 1.25, 0.313, and 0.078 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[Recoverin] (ng/ml)
P1	1 part Standard (20 ng/ml)	20.00
P2	1 part P1 + 3 part EIA Diluent	5.00
P3	1 part P2 + 3 part EIA Diluent	1.25
P4	1 part P3 + 3 part EIA Diluent	0.313
P5	1 part P4 + 3 part EIA Diluent	0.078
P6	EIA Diluent	0.00

- **Biotinylated Recoverin Antibody (100x):** Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent Concentrate 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

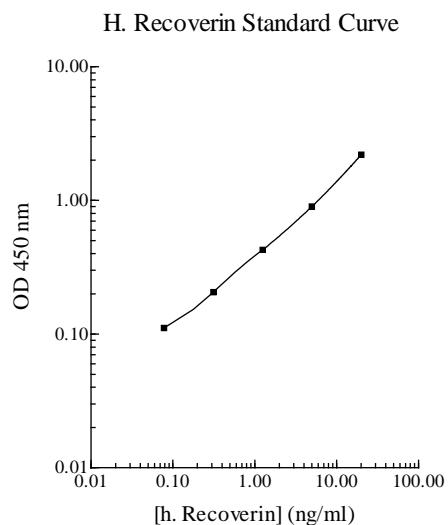
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- Add 50 µl of Biotinylated Recoverin Antibody to each well and incubate for 2 hours.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or 4-parameter curve fit.
- Determine the unknown sample concentration from the Standard Curve.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of recoverin is typically 50 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.2% respectively.
- No significant cross-reactivity or interference was observed.

References

1. Higgins MK *et al.* (2006) *J Biol Chem.* 14;281(28):19426-32
2. ARTHUR S *et al.* (1995) *Proc. Natl. Acad. Sci.* Vol. 92, pp. 9176-9180
3. Bazhin AV *et al.* (2007) *Cancer Immunol Immunother.* Jan;56(1):110-6

Revision 1.2