# Human Recoverin ELISA Kit

Enzyme Immunoassay for the quantification of Human Recoverin in Human Serum, plasma, cell lysates and cell culture supernatants.

Catalog number: ARG82036

Package: 96 wells

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## INTRODUCTION

This gene encodes a member of the recoverin family of neuronal calcium sensors. The encoded protein contains three calcium-binding EF-hand domains and may prolong the termination of the phototransduction cascade in the retina by blocking the phosphorylation of photo-activated rhodopsin. Recoverin may be the antigen responsible for cancer-associated retinopathy. [provided by RefSeq, Jul 2008]

Seems to be implicated in the pathway from retinal rod guanylate cyclase to rhodopsin. May be involved in the inhibition of the phosphorylation of rhodopsin in a calcium-dependent manner. The calcium-bound recoverin prolongs the photoresponse. [UniProt]

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Recoverin has been pre-coated onto a microtiter plate. Human Recoverin standards or samples are pipetted into the wells and any Recoverin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Recoverin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Recoverin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The

concentration of Recoverin in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit as Storage information below. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody-coated microplate | 8 X 12 strips | 4°C. Unused strips should be sealed tightly in the air-tight pouch. |
| Standard | 1 X 80 ng (lyophilized) | 4°C (store at -20°C after reconstitution) |
| Antibody conjugate concentrate (50X) | 1 vial (120 µl) | -20°C |
| HRP-Streptavidin concentrate (100X) | 1 vial (80 µl) | -20°C |
| Dilution Buffer concentrate (10X) | 30 ml | 4°C |
| Wash Buffer Concentrate (20X) | 2 X 30 ml | 4°C |
| TMB substrate | 7 ml (Ready to use) | 4°C |
| STOP solution | 11 ml (Ready to use) | 4°C |
| Plate sealer | 3 strips | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store 50X Antibody conjugate concentrate and 100X Streptavidin-HRP concentrate at-20°C.
- Store Standard at 2-8°C before reconstituting with Dilution Buffer and at -20°C after reconstituting with Dilution Buffer.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Briefly spin down the standards and solutions before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- If crystals are observed in the 20X Wash buffer or 10X Dilution Buffer, warm to RT and mix gently until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before opening and using the kit, spin tubes and bring down all

components to the bottom of tubes.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

a) Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect supernatants. Sample may be assayed directly without dilution, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

b) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and collect serum. Sample may be assayed directly without dilution, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be aliquoted and stored at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

c) Cell Culture Supernatants: Collect cell culture media and centrifuge at 1500 rpm for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be aliquoted and stored at-80°C. Avoid repeated freeze-thaw cycles.

d) Cell Lysate: Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (10 mM Tris pH 8.0, 130 mM NaCl, 1% Triton X-100, and protease inhibitor cocktail). For every 1 x $10^6$ cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. Samples can be stored at-80°C. Avoid repeated freeze-thaw cycles.

e) Tissue Extract: Tissue: Extract tissue samples with 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 1% Triton X-100. Lysis samples by homogenization or by sonication on ice. Then centrifuge at 14,000 rpm for 20 min at 4°C. Collect the supernatant and measure the protein concentration. Aliquot and store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Note:

- Applicable samples may also include other biofluids, and tissue lysates. If necessary, it is recommended to do pre-test to determine the suitable dilution factor.

- Dilution Note (For duplicate):

a) For 100X dilution: add 5 µL of samples into 495 µL of 1X Dilution Buffer, mix well.

b) For 10,000X dilution: add 5 µL of diluted samples from a) into 495 µL of 1X Dilution Buffer, mix well.

c) For 100,000X dilution: add 20 µL of diluted samples from b) into 180 µL of 1X Dilution Buffer, mix well.

## REAGENT PREPARATION

*Freshly dilute all reagents and bring all reagents to room temperature before use.*

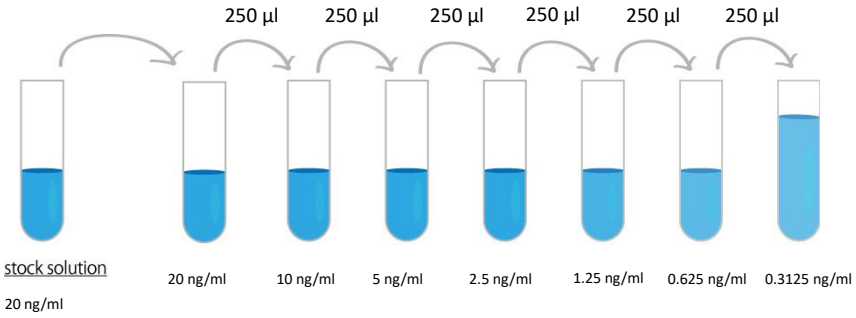
- 1X Dilution Buffer: Dilute 10X Dilution Buffer concentrate into distilled water to yield 1X Dilution Buffer (E.g. 10 ml of 10X Dilution Buffer + 90 ml of distilled water). If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use. The diluted 1X Diluent can be stored for up to 30 days at 2-8°C.
- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 10 ml of 20X wash buffer + 180 ml of distilled water) If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use.
- 1X Antibody conjugate: It is recommended to prepare this reagent immediately prior to use. Briefly spin down the 50X antibody conjugate concentrate. Dilute 50X antibody conjugate concentrate into 1X Dilution Buffer to yield 1X detection antibody solution. (e.g. 60 µl of 50X antibody conjugate concentrate + 2940 µl of 1X Dilution Buffer). Any remaining solution should be frozen at-20°C.
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use. Spin down the 100X HRP-Streptavidin Solution concentrate briefly and dilute the desired amount of the conjugate with 1X Dilution Buffer (E.g. 40 µl of Streptavidin-HRP conjugate + 3960 µl of 1X Dilution Buffer). Any remaining solution should be frozen at-20°C.
- Sample: If the initial assay found samples contain Recoverin higher than the highest standard, the samples can be diluted with 1X Dilution Buffer

and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

(It is recommended to do pre-test to determine the suitable dilution factor).

- Standards: Reconstitute the standard with 4 ml of 1X Dilution Buffer to yield a stock concentration of 20 ng/ml. Allow the stock standard to sit for 10 minutes at room temperature (20-25°C) with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The 1X Dilution Buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted with 1X Dilution Buffer as according to the suggested concentration below: 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml.

  Note: Any remaining stock solution should be aliquoted and stored at -20°C and used within 2 days. Avoid repeated freeze-thaw cycles.



Dilute Recoverin standard as according to the table below:

| Standard | Recoverin Conc. (ng/ml) | µl of Dilution Buffer | µl of standard |
|---|---|---|---|
| S7 | 20 ng/ml | 0 | 500 (20 ng/ml Stock) |
| S6 | 10 ng/ml | 250 | 250 (S7) |
| S5 | 5 ng/ml | 250 | 250 (S6) |
| S4 | 2.5 ng/ml | 250 | 250 (S5) |
| S3 | 1.25 ng/ml | 250 | 250 (S4) |
| S2 | 0.625 ng/ml | 250 | 250 (S3) |
| S1 | 0.3125 ng/ml | 250 | 250 (S2) |
| S0 | 0 | 250 | 0 |

## ASSAY PROCEDURE

Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (RT, 20-25°C). When diluting samples and reagents, they must be mixed completely and evenly. Standard Recoverin detection curve should be prepared for each experiment. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. The remaining microplate strips may be stored for up to 30 days in a vacuum desiccator.
2. Add **50 µl** of **standards, samples and zero control**s (S0, Dilution Buffer) into wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that

may have formed.

3. Cover wells with a sealing tape and incubate for **2 hours at RT (20-25°C)**. Start the timer after the last addition.
4. Remove sealer from plate.
5. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes** (If a microplate washer is used, wash the wells for a total 6 washes). Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **50 µl** of **1X Antibody conjugate** into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
7. Reseal the plate with sealer. Incubate for **2 hour at RT**.
8. **Wash as** according to **step 5**.
9. Add **50 µl** of **1X HRP-Streptavidin Solution** into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
10. Reseal the plate with sealer. Incubate for **30 minutes at RT.** (Turn on the microplate reader and set up the program in advance.)
11. **Wash as** according to **step 5**.
12. Add **50 µl** of **TMB substrate solution** into each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed.
13. Incubate for **15 minutes at RT** or until the optimal blue color density develops. (Protect from light)
14. Add **50 µl** of **STOP solution** into all wells to stop the reaction. The color of

the solution should change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.

15. Read the OD with a microplate reader at **450 nm** immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. 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. So it is recommended read the absorbance **within 10 min** after adding STOP solution.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

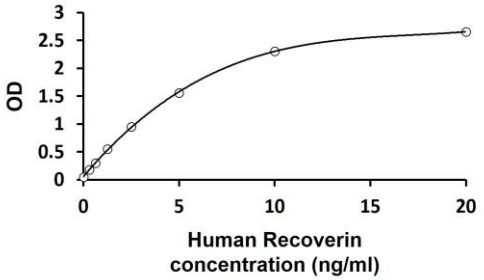
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

The minimum detectable dose (MDD) of Human Recoverin ranged from 0.313 - 20 ng/ml. The mean MDD was 0.09 ng/ml.

### Recovery

85-112%

### Intra-assay and Inter-assay precision

The CV values of intra-assay was 5.1% and inter-assay was 10%.