NEUROMICS

# ELISA Kit for the Sensitive Detection of UCHL1

**Catalog Number:** EL22001

**Species Reactivity:** Human, horse, cow, pig, rat, mouse

**Applications:** ELISA of blood, CSF, urine, and tissue extracts.

**Storage:** Shipped on ice. Store at 4°C.

## Application Notes

**Description:**
Ubiquitin C-terminal hydrolase 1 (UCHL1) was independently discovered by several different research groups and so has several other names, such as ubiquitin carboxyl esterase L1, ubiquitin thiolesterase, neuron-specific protein Pgp9.5 and Park5. It was originally identified as a major neuron specific cytoplasmic protein from 2dimensional gel analysis of brain tissues and immunostaining, and was given the name “protein gene product 9.5” or Pgp9.5.

This assay was developed and used to show that UCHL-1 was released in large amounts into the CSF of aneurismal subarachnoid hemorrhage patients, larger amounts indications both acute events and poorer outcomes. It has since been used to study work from other groups suggesting that blood UCHL-1 is a potentially valuable biomarker of CNS damage and disease states.

**Principles of the Assay:**
ELISA provides a conventional method for measuring the level of protein targets within a single, small-volume sample. EL22001 is a 2-site sandwich enzyme linked immunoassay. Neuromics provides a provides a plate pre-coated with capture mouse monoclonal antibody against UCKL-1. The user incubates the samples on the plate and any UCHL-1 protein in the sample binds to the capture antibody. The user then incubates the plate with a solution containing rabbit polyclonal UCHL-1 detection antibody, which binds to the complex of antigen and captured Ab. The detection antibody is then itself bound by goat anti-rabbit-Horse Radish Peroxidase (HRP) conjugates antibodies. The suer adds a substrate TMB solution which produces a blue colored product in wells containing HRP. The intensity of the color corresponds to the amount of UCHL-1 in the sample. Finally, the user stops the reaction by adding $H_2SO_4$. The color changes from blue to yellow and can be quantified by measurement on a suitable ELISA plate readers using an absorbance wavelength of 450 nm.

**Reagents Supplied:**

| Kit component | Kit component | Quantity | Storage |
|---|---|---|---|
| **Plate** | Pre-coated anti-UCHLA-1 strip plate | 8x12 wells | 4°C |
| **UCHL-1 Standard** | Human recombinant UCHL-1 protein, lyophilized | 2 vials | 4°C |
| **RPCA-UCHL01** | Detection, anti-UCHL-1, rabbit polyclonal antibody (1,000X) | 1 x 20 uL | -20°C |
| **a-Rb-HRP** | Goat anti-rabbit IgG, HRP conjugated antibody (100X) | 1 x 250 uL | -20°C |
| **Block** | Blocking protein | 1 x 1 g | 4°C |
| **TBS** | 1XTBS, ready to use rinsing plate solution | 1 x 12 mL | 4°C |
| **TBST** | 10XTBS – Tween concentrate | 1 x 12 mL | 4°C |
| **TMB** | 1XTMB (Tetramethylbenzidine) substrate ready to use solution | 1 x 12 mL | 4°C |

**Additional Materials/Equipment:**

- Microplate reader (450 nm)
- Microplate shaker
- Automated plate washer
- Multichannel pipette
- Appropriately sized tubes for reagent preparation
- Polypropylene microcentrifuge tubes for preparing dilutions
- Stop solution (2N $H_2SO_4$)
- Wash buffer (1X TBST)

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**Limitations of the Assay:**

- For research use only. Not for use in diagnostic procedures
- Absorbance values beyond the range of the standard curve are not valid.

**Note:** Before starting the assay, read the instructions for use completely and carefully. Make sure that everything is understood.

**Best Practices + Technical Hints:**

1. Do not mix substitute reagents from different sources or different kit lots.
2. Dilute samples in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution. Two common pipetting methods used for ELISA are standard (forward) and reverse. Use standard (forward) pipetting for the preparations of sample dilutions, and reverse pipetting for the addition of diluted samples, controls, and reagents. Careful pipetting is crucial for accurate test results. Become familiar with the pipette and both methods before running actual tests. Be sure to use the correct pipette and tip (volume capacity) for the volume being transferred.
3. Standard (Forward) Pipetting:
    a. Put a new tip on a single-channel pipette and make sure that it is on tight.
    b. Press the plunger to the first stop.
    c. Draw the calibrated column of sample into the tip and pause for one second with the tip still in the sample. Be careful not to place the tip too deeply into the sample.
    d. Touch the tip to the side of the sample container to remove any excess liquid on the outside of the tip.
    e. Dispense the sample into the measured diluent by depressing the plunger past the first stop to the second stop. Be careful not to place the tip too deeply into the sample diluent.
    f. After dispensing the sample into the diluent, rinse the pipette tip in the diluent by pushing the plunger up and down 2-3 times before ejecting the tip.
    g. Mix samples using vortex if they are in the tube and tapping the plate if the samples added directly to the well.
    h. Eject the tip into a waste container.
4. Reverse pipetting using single or multichannel pipette:
    a. Put new tips on the pipette. Make sure they are on tight and straight.
    b. Press the plunger past the first stop and halfway to the second stop.
    c. Draw the liquid in a slow motion, being careful not to draw any air bubbles into the tips, check for consistency of volume in the tips.
    d. Touch the tips to the edge of the reagent reservoir to remove excess liquid on the outside of the tips. If the wells on your plate are empty, position the tips into the lower corner of each well, contacting the plastic. If the wells on your plate contain liquid, contacting the plastic.
    e. Slowly dispense the liquid into the wells by depressing the plunger to the first stip. Be careful not to splash liquid out of the wells and make sure there are no drops left on the tips.
    f. To repeat, hold the plunger at the first stop and continue with step “c”.
    g. Eject the tips into a waste container.

    **Note:** Reverse pipetting uses more reagent/volume (=”dead volume”)
5. Measure desire amounts of reagents by weighing or pipetting them from original container.
6. Avoid prolonger exposure of HRP-conjugates antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
7. Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results and are particularly a problem during optical density determination.
8. When using an automated plate washer, rotating the plate 180 degrees between washing steps may improve assay precision.
9. Gently tap the plate against paper towel to remove residual fluid after washing.
10. The TMB developer solution should be at **room temperature** when added to the plate. Keeping time intervals consistent between adding developing buffer and reading the plate should improve inter-plate precision.
11. If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the wells. If assay results are above the top of the calibration curve, dilute samples, and repeat the assay.

**Reagent Preparation:**

1. Wash Solution – TBST
    a. Wash solution is 1X tris buffered saline – tween (TBST) (10mM Tris base, 150 mM NaCl, 0.1% Tween-20), for 1L
        i. 1.21 g – Tris base
        ii. 9.77g – NaCl
    b. Add deionized water to a final volume of 900 mL

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c. Adjust pH to 7.5 using concentrated HCl (careful!), then add 1 mL – Tween 20, mix well.
d. Bring final volume to 1L with $dH_2O$.

2. Stop Solution
   a. Stop solution is 2N $H_2SO_4$ solution to make 250 mL
      i. 26.7 mL concentrated $H_2SO_4$
      ii. 223.3 mL $H_2O$
   b. Stop solution can be stored at room temperature for up to 3 months.
3. Dilution Buffer (DB)
   a. Weigh out 500 mg of blocker and combine it with 5 mL of 10X TBST (provided by Neuromics), add $dH_2O$ to have final volume of 50 mL, dissolve well. The buffer can be stored at 4°C for 2 days.
4. Dilute Detection Antibody
   a. Prepare the detection antibody solution immediately prior to use.
   b. Neuromics provides detection, rabbit polyclonal UCHL-1 antibody as 1,000X stock solution, The working solution is 1X. For 1 plate combine:
      i. 12 mL of dilution buffer (DB)
      ii. 12 uL of detection antibody, vortex well
   c. Make enough solution for the experiment, and discard remainders after use.
5. Dilute a-Rb-HRP Antibody
   a. Prepare the a-Rb-HRP antibody solution immediately prior to use.
   b. Neuromics provides Goat anti-rabbit IgG, HRP conjugates antibody as 100X stock solution. The working solution is 1X. For 1 plate combine:
      i. 12 mL of dilution buffer (DB)
      ii. 120 uL of a-Rb-HRP, vortex well
   c. Make enough solution for an experiment and discard remainders after use.

**Sample Preparation:**

Serum and Plasma:
Plasma samples prepared in heparin tubes commonly display additional clotting following thawing. Remove clots and all solid material by centrifugation. Similarly, serum may sometimes contain small amounts of particulate material which can be removed by centrifugations. For those systems and assays using neat samples or lower dilution factors, the sample can be put directly into the wells of the coated plates. Follow the sequence below:

1. Add the dilution buffer (DB) to the plate.
2. Add the sample into the DB.
3. Mix by tapping the plate or repeating pipetting.

We recommend starting with diluted plasma or serum samples 1:2.5 times with DB (or 30 uL of dilution buffer plus 20 uL of serum or plasma sample) at a single concentration for pilot experiments. If strong UCHL-1 signals are detected samples may run at higher dilutions. Always run samples in duplicate or triplicate. This will provide enough data for statistical validation of the results.

CSF Samples:
Pathological human CSF samples are generally higher in UCHL-1 concentration. Start pilot experiments with 1:5 dilution of CSF in DB (in order to dilute samples directly in the plate, add 40 uL of dilution buffer to the well then add 10 uL of CSF samples, mix by tapping the plate). If strong signals are detected samples may be diluted more (dilute CSF samples in the tube, then add 50 uL to the well). Always run samples in duplicate or triplicate. This will provide enough data for statistical validation of the results.

**Protocol:**

1. Remove the plate from a plastic bag. It is ready to use.
2. Rehydrate the plate with 1XTBS (provided). Add 100 uL of the solution and aspirate it.
3. Designate two or three columns of the plate for the standard curve. Perform standard curve (see "Protein Standards Preparation"). **Note:** A standard curve must be prepared for each plate!
4. Add 50 uL of diluted samples per well or dilute them directly in the wells (see "Sample preparation"). Incubate the plate for at least 2 hours at room temperature with gentle shaking, for better performance overnight at 4°C (Seal the plate).
5. Wash the plate 2X3 times with 300 uL of TBST using an automated plate washer, rotate the plate 180 degrees between wash steps.
6. Add 100 uL of detection antibody solution (RPCA-UCHL1, 1:1000 dilution, or 12 mL of DB + 12 uL of RPCA-UCHL1) to each well. Incubate the plate for at least 2 hours at room temperature, or overnight at 4°C with dentle shaking (Seal the plate).

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7. Wash the plate 2X3 times with 300 uL of TBST using an automated plate washer, rotate the plate 180 degrees between wash steps.
8. Add 100 uL of a-RB-HRP (1:100 dilution, or 12 mL of DB + 120 uL of a-Rb-HRP) to each well. Incubate the plate for 1-1.5 hours at room temperature with gentle shaking.
9. Bring TMB solution to room temperature.
10. Wash the plate 2X3 times with 300 uL of TBST using an automated plate washer, rotate the plate 180 degrees between wash steps.
11. Add 100 uL of TMB (the solution should be at Room Temperature) developing solution. Incubate the plate until you will reach a desired color change; the highest standard should be dark blue, while Blank should stay colorless. Time of incubation varies from 5-20 minutes (average time is 10 minutes).
12. Stop reaction with 50 uL of stop solution; 2N $H_2SO_4$. Note, that the color changes from blue to yellow. No incubation in stop solution is required before reading the plate.
13. Read the plate on the plate reader-spectrophotometer at 450 nm. There are many models and manufacturers of plate readers; refer to the manufacturer's instructions for details of operation.

**Performance Characteristics of the UCHL-1 ELISA**

Measuring Range:
The standard curve covers the interval 0.156 – 10 ng/ml UCHL1. Extrapolation beyond the curve with not provide accurate results. Samples displaying signal outside the curve should be further diluted and remeasured.

Detection Limit:
The least detectable concentration is 0.159 pg/mL, defined as the mean of blank samples + 2 STDEV.

Sensitivity:
The intra-assay variability %CV < 6. The inter-assay variability %CV < 10

Shelf Life:
When stored at an appropriate temperature, the product can be used within 6 months from date of purchasing.

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