UCHL1 ELISA Kit

Enzyme Immunoassay kit for the quantification of UCHL1 in plasma, serum and CSF samples.

Catalog number: ARG81165

Package: 96 wells

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INTRODUCTION

The protein encoded by UCHL1 gene belongs to the peptidase C12 family. This enzyme is a thiol protease that hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. UCHL1 gene is specifically expressed in the neurons and in cells of the diffuse neuroendocrine system. Mutations in UCHL1 gene may be associated with Parkinson disease. [provided by RefSeq, Sep 2009]

Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins. This enzyme is a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. Also binds to free monoubiquitin and may prevent its degradation in lysosomes. The homodimer may have ATP-independent ubiquitin ligase activity. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of UCHL1 in plasma, serum and CSF samples. UCHL1 in samples and standards will bind to the capture mouse monoclonal antibody coated on the microtiter plate. After appropriate washing steps, anti-UCHL1 polyclonal primary antibody binds to the captured protein. Following a washing to remove unbound substances, secondary antibody 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 UCHL1 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 UCHL1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 strips X 12 wells	4°C
Standards	2 vials	4°C
Anti-UCHL1 Primary Antibody (1000X)	20 µl	-20°C
HRP-conjugated Secondary antibody (100X)	250 µl	-20°C
Blocker	1 g	4°C
TBS	12 ml (ready-to-use)	4°C
10X TBST	12 ml	4°C
TMB substrate	12 ml (ready-to-use)	4°C (Protect from light)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 2N H_2SO_4 for stopping color development
- 1X TBST for washing
- Microplate shaker (shaking amplitude 3 mm; approx. 300 rpm), or an orbital shaker. If other kind of shaker is used, the rotating speed may need to be optimized by user.
- 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.
- This kit can be stored for up to 6 months. All components should be stored at 4°C except the Primary Antibody and Secondary Antibody.
- Briefly spin down the Primary, Secondary Antibody and standard before use.
- 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.

SAMPLE COLLECTION & STORAGE INFORMATION

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

The kit measures UCHL1 in mammalian CSF, serum and plasma. Mix thawed samples thoroughly just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at-20°C or store at -70°C for long-term storage. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at-20°C or store at -70°C for long-term storage. Avoid repeated freeze-thaw cycles.

CSF: CSF samples should be aliquoted and stored at-70°C. Avoid repeated freeze-thaw cycles.

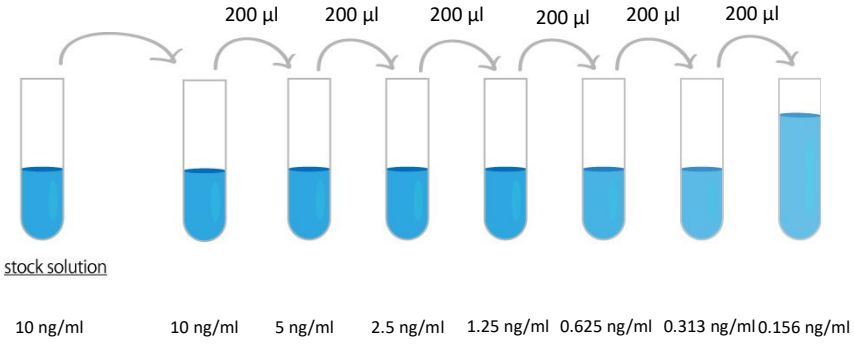
REAGENT PREPARATION

- **Dilution Buffer:**

- **1X TBST:** Dilute 5 ml of 10X TBST buffer (supplied in the kit) into 45 ml of distilled water to yield 50 ml of 1X TBST buffer.
- **Dilution Buffer**: Add 0.5 g of Blocker into 50 ml of 1X TBST (diluted from 10X TBST as above step) and mix well. The reconstituted Dilution Buffer can be stored at 4°C for 2 days.

- **TMB substrate:** The solution should be stored at 4°C. For best results, bring the reagent to room temperature prior to use.
- **Samples** If the measuring absorbance of samples is higher than the highest standard, dilute the samples with Dilution Buffer before assay and assay again. A 1:2.5 dilution for serum/plasma/CSF samples is suggested for the starting dilution. (E.g. 40 µl sample + 60 µl Dilution Buffer). For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standard**: Reconstitute the standard with **400µl** standard diluent buffer to yield a stock concentration of 10 ng/ml. Keep the buffer in the vail for at least 15 min at RT to make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng /ml, 0.625 ng/ml, 0.313 ng/ml, 0.156 ng/ml**. DO NOT reuse the reconstituted standard.



- **1X TBST for washing**: Prepare 1X TBST wash buffer: **0.01M Tris base**, **0.15M NaCl**, **pH 7.5** (Adjust by concentrated HCl). Add Tween-20 into 1x TBS buffer to yield the final concentration of **Tween-20 at 0.1%**.

- **Primary Antibody:** 20 minutes before use, briefly centrifuge vial before opening. Dilute anti-UCHL1 Primary antibody at **1:1000** in **Dilution Buffer** (E.g.: add 10 µl of anti-UCHL1 Primary antibody in to 10 ml of Dilution Buffer). Only prepare amount needed, and discard remainders after use.

- **HRP-conjugated Secondary antibody:** 20 minutes before use, briefly centrifuge vial before opening. Dilute HRP-conjugated secondary antibody at **1:100** in **Dilution Buffer** to yield a 1X working HRP-conjugated secondary antibody, mix thoroughly. (E.g.: Dilute 250 µl of HRP-conjugated secondary antibody in 25 ml of Dilution Buffer.) Only prepare amount needed, and discard remainders after use.

ASSAY PROCEDURE

TMB substrate should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** per well of **standards** or **diluted samples** in duplicates into appropriate wells. Cover wells and incubate the plate at RT for **2.5 hours at 37°C** or **overnight at 4°C**.
3. Aspirate each well and wash, repeating the process 5 times for a total 6 washes. Wash by filling each well with **1X TBST for washing** (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. 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.
4. Add **100 µl** of **working Primary Antibody** into each well. Cover wells and incubate the plate for **2 .5 hours at RT** or **overnight at 4°C**.
5. Wash as according to step 3.
6. Add 100 µl of working **1X HRP-conjugated secondary Antibody** into each well. Cover wells and incubate the plate for **1.5 hour at RT**.
7. Wash as according to step 3.
8. Add **100 µl** of **TMB substrate** to each well. Cover wells and incubate **5-20 minutes at RT** in dark.
9. Add **50 µl** of **2N H_2SO_4** to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking/tapping the plate.

Take care to avoid creating bubbles which will create a strong interfering absorbance signal.

10. Read the OD with a microplate reader at 450 nm immediately. It is recommended read the absorbance within 3 min after adding STOP solution.

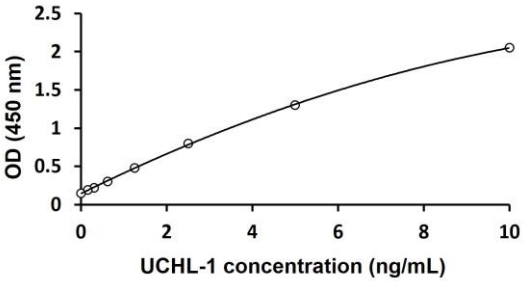
CALCULATION OF RESULTS

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. Use 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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above. If following the dilution suggestion as the protocol as above the dilution factor will be 2.5. So the measured concentration of samples calculated from the standard curve must then be multiplied by 2. E.g. 400 pg/ml (from standard curve) x 2.5 (dilution factor) = 1000 pg/ml.

6. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

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

Standard Range

The standard range can cover form 0.156– 10 ng/ml.

Sensitivity

0.083 pg/ml

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was < 6%

inter-assay precision was < 10%.