Human Cthrc1 ELISA Kit (Do-It-Yourself)  
(Catalog#: hCthrc1-ELISA)  
(U.S. Patent No. 9,718,878)

1. **Product Data Sheet**

**Human Cthrc1 ELISA Kit (Do-It-Yourself)** contains the key components for quantification of human Cthrc1 in biological fluids (e.g. plasma) and cell culture supernatants by sandwich ELISA.

**Components:**
- One vial capture antibody (Vli13E09, 50µg) sufficient for approximately three 96 well plates
- One vial of human Cthrc1 expressed in CHO-K1 cells (500ng, dehydrated, contains Tween20 and sucrose)
- One vial of detection antibody biotinylated conjugate (Vli10G07-biot, 50µg), dehydrated

**Description:**
- Sandwich ELISA using two monoclonal affinity-purified antibodies
- Detects human Cthrc1, not reactive with rat or mouse Cthrc1
- Lower limit of detection 0.3pg/ml
- Protocol included

**To be provided by user:**
- ELISA plates/strips (e.g. Nunc Maxisorp, flat bottom)
- Bicarbonate/carbonate buffer (100mM, pH9.6),
- PBS-T wash buffer with 0.1% bovine serum albumin
- Blocking Buffer (recommended: General Block, #632, immunochemistry.com)
- Streptavidin (SA)-HRP Conjugate (recommended: Vector Laboratories, SA-5004)
- TMB substrate and phosphate/citrate buffer (recommended: TMB PLUS LIQUID 1-Component Substrate, Amresco K8: 1-Step™ Ultra TMB-ELISA, Thermo Scientific Prod # 34028)
- Stop solution (2M sulfuric acid)
- Microplate reader
- Microfuge tubes

**Application Notes:**
Due to the proteolytic susceptibility of Cthrc1, the assay should be preferably performed on plasma samples. Cthrc1 aggregates and addition of Tween-20 (0.2% final concentration) to plasma prior to freezing is highly recommended. 100µl plasma is required for a single measurement. Sample dilution may be necessary for samples with high levels.

**References:**

**ELISA on human plasma.** A sandwich ELISA for human Cthrc1 is shown with 88 different plasma samples. A standard curve with recombinant Cthrc1 is shown in the first column.
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Human Cthrc1 ELISA Kit (Do-It-Yourself) contains the key components for quantification of human Cthrc1 in biological fluids (e.g. plasma) and cell culture supernatants by sandwich ELISA.

Kit Reagents Provided: One vial capture antibody (Vli13E09, 50µg, reconstitute as indicated on vial and store at -20°C, sufficient for three 96 well plates), one vial of dehydrated recombinant human Cthrc1 (500ng, expressed in CHO-K1 cells, store at -20°C) as standard, biotinylated detection antibody (Vli10G07-biotin, 50µg, reconstitute as indicated on vial and store at -20°C).

Materials and Solutions Required but NOT Included:
- ELISA plates/strips (e.g. Nunc Maxisorp, flat bottom)
- Bicarbonate/carbonate buffer (100mM, pH9.6),
- Buffer A: PBS containing 0.1% BSA, 0.1% Tween 20
- Blocking Buffer: General Block (cat. #632, immunochemistry.com, recommended), or Buffer A
- Substrate Solution: TMB (e.g. 1-StepTM Ultra TMB-ELISA, ThermoFisher Scientific Prod # 34028, TMB PLUS LIQUID 1-Component Substrate, Amresco K830)
- Stop Solution: 2N H2SO4
- Streptavidin-HRP Conjugate: e.g. Streptavidin-HRP Concentrate (Vector Laboratories, SA-5004)
- Tubes: polypropylene

Microplate Shaker and Microplate Reader (450nM)

Sample dilution for assay: Plasma samples with high Cthrc1 levels may require dilution of up to 1:10.

Sandwich ELISA Protocol:
1. Coat Maxisorp Nunc ELISA strips/plates with 100µl/well of reconstituted 13E9 IgG (1.8µg/ml final) in bicarbonate/carbonate buffer (100mM, pH9.6), overnight at 4°C, with lid and inside a plastic bag to prevent evaporation.
2. The next morning wash 3 times with 400µl/well of Buffer A.
3. For blocking, load 400µl of General BLock per well, incubate for ≥120min on shaker at RT.
4. Prepare standard by making serial dilutions of the dehydrated purified hCthrc1 in Buffer A. The total amount of the recombinant, lyophilized hCthrc1 is specified on the vial. The dilutions should typically cover a range from 125ng/ml to 0.005ng/ml. Include blank wells.
5. Remove blocking solution (Buffer A) from well one column at a time (suctioning works well), and load standard (100µl/well) and samples (100µl/well). Incubate for 2h at RT on microplate shaker.
6. Wash 4 times with 400µl/well Buffer A.
7. Add 100µl/well of reconstituted Detection Antibody (Vli10G07-biotin, diluted 1:500 in Buffer A and incubate for 1h at RT on microplate shaker.
8. Wash all wells 4 times with 400µl/well Buffer A.
9. Add 100µl/well Streptavidin-HRP (1:250) in Buffer A and incubate for 30 min at RT on microplate shaker.
10. Wash 4 times with 400µl/well Buffer A.
11. Add 100µl/well of TMB Substrate Solution and incubate for 5-10 min on microplate shaker. Blank wells should not turn blue.
12. Add 50µl/well Stop Solution and read optical density at 450nm.

Determine Cthrc1 Concentrations from Standard Curve:
Create a standard curve from the readings of the calibration curve using computer software (e.g. http://www.graphpad.com/faq/file/P4-Nonlinear%20standard%20curves.pdf). If applicable, consider dilution factor in the calculation of Cthrc1 sample concentration.