



ab123453

**CTIP2 (BCL11B) Human
ELISA Kit**

Instructions for Use

For the quantitative measurement of Human CTIP2 protein.

This product is for research use only and is not intended for diagnostic use.

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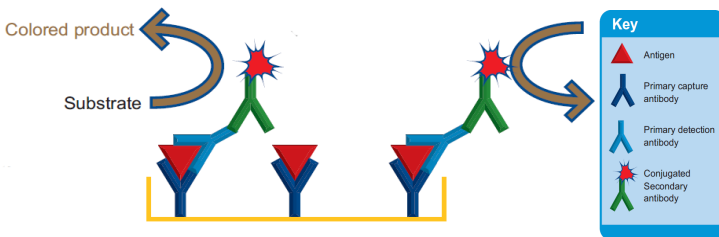
ab123453 CTIP2 Human ELISA Kit

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2. Introduction

Principle: ab123453 CTIP2 Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human CTIP2 in cell and tissue lysates. The assay employs an antibody specific for Human CTIP2 coated onto 96-well plate strips. Standards and samples are pipetted into the wells and the analyte present in the sample is bound to the wells by the immobilized antibody. The wells are washed and an anti-CTIP2 primary detector antibody is added. After washing away unbound primary detector antibody, HRP-conjugated secondary detector antibody specific for the primary detector antibody is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of analyte bound. The developing blue color is measured at 600 nm. Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.



Background: Ctip2 (encoded by BCL11B or CTIP2 gene) is a C2H2-type zinc finger protein and is closely related to Ctip1. Over expression of these proteins has been linked to B cell malignancies. Ctip2 shows a very high level of expression in layer V in both corticospinal motor neurons (CSMN) and corticotectal neurons. Studies have shown that CTIP2 has critical roles in the immune system, controlling T cell subtype specification and survival in the developing thymus. Ctip2 plays a critical role in the development of CSMN axonal projections to the spinal cord in vivo. ChIP assays have revealed that expression of CTIP2 in mammalian cells resulted in deacetylation of histones H3 and/or H4 that were associated with the promoter region of a reporter gene. SIRT1, a class III histone deacetylase, was found to interact directly with CTIP2 and was recruited to the promoter template in a CTIP2-dependent manner. CTIP2 is also a regulator of the development of skin and ameloblast differentiation and in tooth development.

3. Assay Summary

Bring all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



Add 50 μ L standard or sample to each well used. Incubate 2 hours at room temperature.



Aspirate and wash each well two times. Add 50 μ L prepared 1X Detector Antibody to each well. Incubate 1 hour at room temperature.



Aspirate and wash each well two times. Add 50 μ L prepared 1X HRP Label to each well. Incubate 1 hour at room temperature.



Aspirate and wash each well three times. Add 100 μ L TMB Development Solution to each well. Immediately begin recording the color development with elapsed time for 15 minutes at 600 nm. Alternatively add a Stop solution at a user-defined time and read at 450 nm.

4. Kit Contents

Item	Quantity
20X Buffer	20 mL
Extraction Buffer	25 mL
10X Blocking Buffer	6 mL
TMB Development Solution	12 mL
10X CTIP2 Detector Antibody	1 mL
10X HRP Label	1 mL
Human CTIP2 Standard (1000 ng)	1 vial
CTIP2 Microplate (12 x 8 antibody coated well strips)	96 wells

Storage and Handling

Store all components at 4°C. This kit is stable for at least 6 months from receipt. After reconstitution the standard should be stored at -80°C. Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 600 nm (or 450 nm after addition of Stop solution - not supplied).
- Method for determining protein concentration (BCA assay recommended).
- Deionized water
- Multi and single channel pipettes
- PBS (1.4 mM KH_2PO_4 , 8 mM Na_2HPO_4 , 140 mM NaCl, 2.7 mM KCl, pH 7.3)
- Tubes for standard dilution
- Stop solution (optional) – 1N hydrochloric acid
- Optional plate shaker for all incubation steps
- Wellplate cover or seals

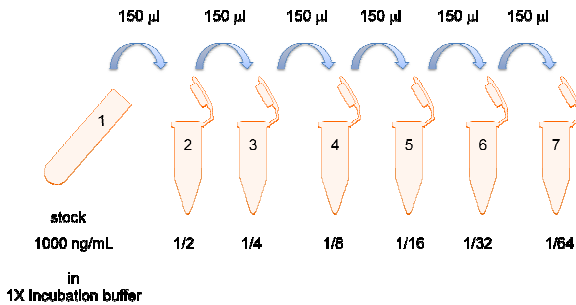
6. Preparation of Reagents

- 6.1 Equilibrate all reagents and samples to room temperature (18-25°C) before use.
- 6.2 Prepare 1X Wash Buffer by adding 20 mL 20X Buffer to 380 mL nanopure water. Mix gently and thoroughly.
- 6.3 Prepare 1X Incubation Buffer by adding 6 mL 10X Blocking Buffer to 54 mL 1X Wash Buffer, mix by gentle inversion. Unused 1X Incubation buffer may be stored at -20°C for 6 months after performing the ELISA.
- 6.4 Prepare the 1X Detector Antibody by diluting the 10X CTIP2 Detector Antibody 10-fold with 1X Incubation Buffer immediately prior to use. Prepare 0.5 mL for each 8 well strip used.
- 6.5 Prepare the 1X HRP label by diluting the 10X HRP Label 10-fold with 1X Incubation Buffer immediately before use. Prepare 0.5 mL for each 8 well strip used.
- 6.6 Reconstitute the standard by adding 1 mL 1X Incubation buffer to the stock Human CTIP2 standard (1000 ng) tube by pipette. Allow to sit for 10 minutes and repeat pipetting to ensure thorough reconstitution. This 1000 ng/mL stock of standard

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material is then used to generate a standard curve in labeled tubes as described below. Any remaining stock material can be stored at -80°C .

- 6.7 To prepare serially diluted standards, label six tubes #2-7. Label the reconstituted stock Human CTIP2 standard from step 6.6 tube #1. Add 150 μL of 1X Incubation buffer to each of tubes #2 through #7. Transfer 150 μL from tube #1 to tube #2. Mix thoroughly. With a fresh pipette tip transfer 150 μL from #2 to #3. Mix thoroughly. Repeat for Tubes #4 through #7. Use 1X Incubation buffer as the zero standard tube labeled #8. Use fresh standards for each assay.



7. Sample Preparation

Note: Extraction buffer can be supplemented with phosphatase inhibitors, PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Cell lysates

- 7.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 10 min at 4°C.
- 7.1.2 Rinse cells twice with PBS.
- 7.1.3 Solubilize cell pellet at 2×10^7 /mL in Extraction Buffer.
- 7.1.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

7.2 Tissue lysates

- 7.2.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- 7.2.2 Suspend the homogenate to 25 mg/mL in PBS.
- 7.2.3 Solubilize the homogenate by adding 4 volumes of Extraction Buffer to a sample protein concentration of 5 mg/mL.
- 7.2.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

The sample should be diluted to within the working range of the assay in 1X Incubation Buffer. As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Data Analysis.

8. Assay Procedure

Equilibrate all reagents and samples to room temperature before use. It is recommended all samples and standards be assayed in duplicate.

- 8.1 Prepare all reagents, working standards, and samples as directed in the previous sections.
- 8.2 Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and seal.
- 8.3 Add 50 μL of each serially diluted Human CTIP2 standard (tubes 2 – 8) or test sample per well. It is recommended to include a dilution series of a control (normal) sample as a reference. Also include a 1X Incubation buffer as a zero standard.
- 8.4 Cover/seal the plate and incubate for 2 hours at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.5 Aspirate each well and wash, repeat this once more for a total of **two** washes. Wash by aspirating or decanting from wells then dispensing 300 μL 1X Wash buffer into each well as described above. Complete removal of

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liquid at each step is essential to good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

- 8.6 Immediately before use prepare sufficient (0.5 mL/strip used) 1X Detector Antibody (step 6.4) in 1X Incubation buffer. Add 50 μ L 1X Detector antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.7 Repeat the aspirate/wash procedure above.
- 8.8 Immediately before use prepare sufficient (0.5 mL/strip used) 1X HRP label in 1X Incubation buffer (step 6.5). Add 50 μ L 1X HRP label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.
- 8.9 Repeat the aspirate/wash procedure above, however, performing a total of **three** washes.
- 8.10 Add 100 μ L TMB Development Solution to each empty well and immediately record the blue color development

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with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nM
Time:	up to 15 min.
Interval:	20 sec. - 1 min.
Shaking:	Shake between readings

(Alternative– In place of a kinetic reading, at a ***user defined***, time record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 100 µL stop solution (1N HCl) to each well and record the OD at 450 nm.

8.11 Analyze the data as described below.

9. Data Analysis

Average the duplicate standard readings and plot against their concentrations after subtracting the zero standard reading. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read CTIP2 protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

TYPICAL STANDARD CURVE - *For demonstration only.*

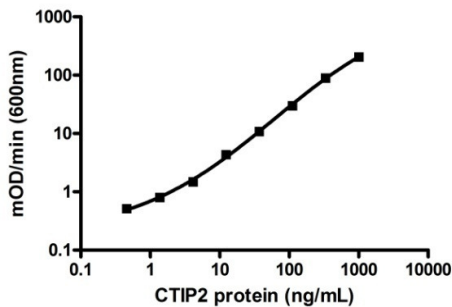


Figure 1. Example standard curve. A dilution series of recombinant CTIP2 in the working range of the assay.

TYPICAL SAMPLE RANGE - *For demonstration only.*

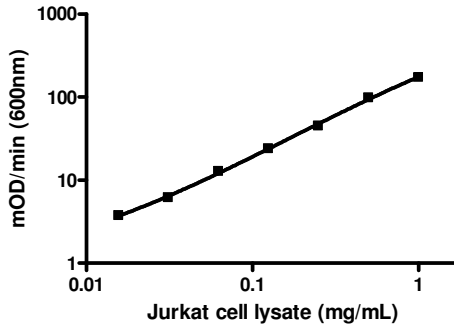


Figure 2. Example curve for endogenous CTIP2 expression in Jurkat cells.

Typical working ranges	
Sample Type	Range
Whole Jurkat cell culture extract	0.015 - 1 mg/mL
CTIP2 standard	2 – 1000 ng/mL

SENSITIVITY

Determined minimum detectable dose = 2 pg/mL CTIP2 standard.

LINEARITY OF DILUTION

Linearity of dilution determined by comparing dilution series of Jurkat extract with standard CTIP2 protein (starting extract concentration is 1 mg/mL).

Sample Type	% Expected Value
1:1	100
1:2	113
1:4	94
1:8	95
1:16	97
1:32	83
1:64	88

REPRODUCIBILITY

Parameter	CV%
Intra (n=8)	9.6
Inter (n=3 days 8 replicates eac)	13.0

RECOVERY

CTIP2 is an intracellular marker, however interference by cell culture media and serum were determined.

Sample Type	Average Recovery (%)	Range (%)
Cell culture media	90	80-105
10% Serum	94	78-117

10. Specificity

Species– Human reactive.

Specificity of this ELISA was confirmed by analyzing various samples from different species known to contain high levels of CTIP2 expression, see Figure 3. The specificity of the CTIP2 detector antibody in this kit was independently confirmed by Western blot, ICC and Flow cytometry, see Figure 4.

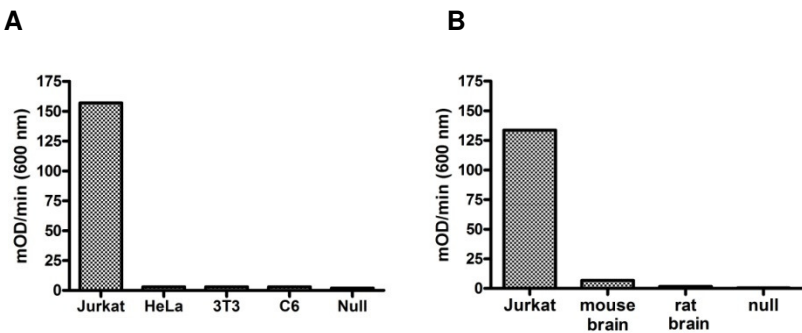


Figure 3. Demonstration of the specificity of this ELISA. (A) Endogenous expression of CTIP2 in Jurkat cells is higher than other non-lymphocyte cells such as HeLa (Human carcinoma) and NIH3T3 (mouse fibroblast), and C6 (rat glioma). (B) CTIP2 expression is detectable in mouse brain lysate but not in rat brain lysate.

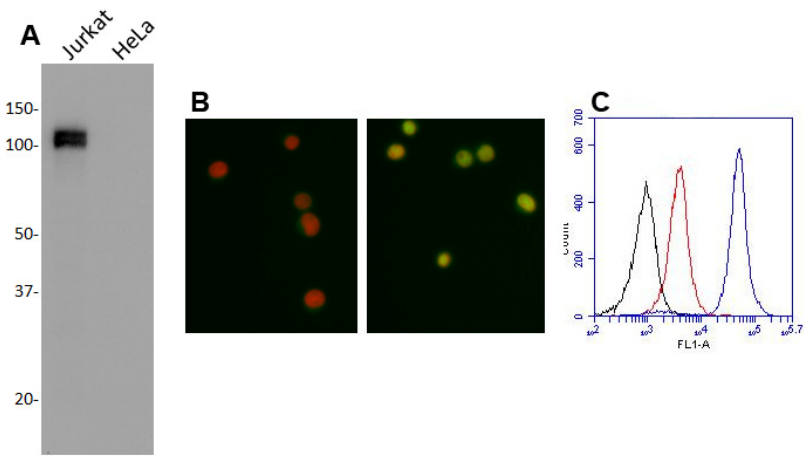


Figure 4. Demonstration of the specificity of the detector antibody used in this kit. (A) Western blot using the CTIP2 detector antibody at 1 μ g/mL: Jurkat cell lysate and HeLa cell lysate, each were loaded at 16 μ g/lane. (B) Analysis of CTIP2 staining in Jurkat cells by ICC: left panel DAPI (red for contrast) with no primary antibody and right panel CTIP2 detector primary antibody at 1 μ g/mL (green), both samples contain 488 labeled secondary antibody. (C) Analysis of CTIP2 staining in Jurkat cells by Flow: Black unstained, red no primary and blue CTIP2 detector antibody at 1 μ g/mL, red and blue include 488 dye labeled secondary.

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80 °C, all other assay components 4 °C. Keep substrate solution protected from light

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