ab284026 – Human CD7 ELISA Kit

For the quantitative measurement of CD7 in human Cell lysate and Tissue Samples.
For research use only - not intended for diagnostic use.

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the SP Conjugate, Standard and Biotinylated Antibody which should be stored at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
Human CD7 Microplate	96 wells	4°C
Sealing Tapes	3	N/A
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
10X Diluent M Concentrate	20 mL	4°C
1X Standard Diluent	2 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
25X Biotinylated Human CD7 Antibody	1 vial	-20°C
Chromogen Substrate	7 mL	4°C
CD7 Standard	1 vial	-20°C
Stop Solution	11 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 nm
Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)
Deionized or distilled reagent grade water

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
Prepare only as much reagent as is needed on the day of the experiment.
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent M Concentrate: Dilute the Diluent M Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.

25X Biotinylated Human S100A16 Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 25-fold with Diluent M to produce a 1x solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.

100X Streptavidin-Peroxidase Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.
Prepare serially diluted standards immediately prior to use.
Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).
Reconstitution of the Human CD7 Standard vial to prepare **20 ng/mL Stock Standard**.

- First consult the Human CD7 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of **Standard Diluent** to add when resuspending the Human CD7 Standard vial to produce 20 ng/mL Stock Standard by using the following equation:
    - C_S = Starting mass of Human CD7 Standard (see vial label) (µg)
    - C_F = The 20 ng/mL CD7 Stock Standard final required concentration
    - V_D = Required volume of Standard Diluent for reconstitution (µL)
    - Calculate total required volume Standard Diluent for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example: **Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided**.

CS = 10 ng of Human CD7 Standard in vial

CF = 20 ng/mL Human CD7 Stock Standard final concentration

VD = Required volume of Standard Diluent for reconstitution (10 ng / 20 ng/mL) x 1,000 = 500 µL

- Reconstitute the Human CD7 Standard vial by adding the appropriate calculated amount VD of **Standard Diluent** to the vial to generate the 20 ng/mL Human CD7 Stock Standard. Mix gently and thoroughly.
- Allow the reconstituted 20 ng/mL Albumin Stock Standard to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label tubes #1 – 8.
- Add 120 µL of **1X Diluent M** to tubes #1 – 8.
- To prepare Standard #1, add 120 µL of the Stock Standard into tube #1 and mix gently.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent M serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	S100A16 (ng/mL)
1	120 µL Stock Standard	120	10
2	120 µL Standard #1	120	5
3	120 µL Standard #2	120	2.5
4	120 µL Standard #3	120	1.25
5	120 µL Standard #4	120	0.625
6	120 µL Standard #5	120	0.313
7	120 µL Standard #6	120	0.156
8	-	120	0.0

Sample Preparation

Cell Lysate: Rinse cell with cold phosphate-buffered saline (PBS) and then scrape the cell into a tube that contains 5 ml of cold PBS with 5 mM EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 106 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. If necessary, dilute samples into EIA Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Tissue: Extract tissue samples with PBS containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into EIA Diluent; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed, and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate. The assay is performed at room temperature (20-25°C).
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.

1. Add 50 µL of Human CD7 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
2. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
3. Add 50 µL of Biotinylated Human CD7 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
4. Wash the microplate as described above.
5. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
6. Wash the microplate as described above.
7. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue colour density develops.
8. Add 50 µL of Stop Solution to each well. The colour will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
9. Read the absorbance on a microplate reader at a wavelength of 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.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

TYPICAL DATA

Provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Point	ng/mL	OD	Average OD
P1	10.0	2.055 1.923	1.989
P2	5.0	1.377 1.291	1.334
P3	2.5	0.782 0.728	0.755
P4	1.25	0.418 0.388	0.403
P5	0.625	0.227 0.209	0.218
P6	0.313	0.128 0.116	0.122
P7	0.156	0.076 0.080	0.078
P8	0.0	0.042 0.044	0.043

PERFORMANCE CHARACTERISTICS

1. The minimum detectable dose of human CD7 as calculated by 2SD from the mean of a zero standard was established to be 86 pg/mL.
2. Intra-assay precision was determined by testing three plasma samples ten times in one assay.
3. Inter-assay precision was determined by testing three plasma samples in ten assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	4.4	10.8

RECOVERY

Standard Added Value	0.625 - 5 ng/mL
Recovery %	95 – 113
Average Recovery %	102

CROSS REACTIVITY

Species	Cross-Reactivity (%)
Human CD14	5%
Human CD84	5%

No significant cross-reactivity observed with CD5, CD27, CD33, CD38, CD40LG, CD47, CD70, CD74, CD79B, CD200, CD207, CD226, and CD244 proteins.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

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