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ab222878
Human VTA1 ELISA Kit

For the quantitative measurement of human VTA1 in cell culture supernatants and cell lysates.

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

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1. Overview

The Human VTA1 ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab222878) is designed for detection of human VTA1 in cell lysates and cell culture samples.

This assay employs a quantitative sandwich enzyme immunoassay technique that measures human VTA1 in less than 4 hours. A polyclonal antibody specific for human VTA1 has been pre-coated onto a 96-well microplate with removable strips. VTA1 in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for VTA1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Vacuolar protein sorting-associated protein VTA1 homolog (VTA1), also known as dopamine-responsive gene 1 protein (DRG-1), LYST-interacting protein 5 (LIP5), and SKD1-binding protein 1 (SBP1), is predominantly a cytosolic protein. VTA1 consists of 307 amino acids with a predicted molecular mass of 34 kDa. It is involved in the endosomal multivesicular body (MVB) formation and trafficking. The MVB is an endosomal compartment that serves to sort membrane proteins destined for degradation or routing to the lysosome. These membrane proteins, such as stimulated growth factor receptors, lysosomal enzymes and lipids, are internalized into intraluminal vesicles that are generated by the invagination and scission from the limiting membrane of the endosome. The contents of the MVB are then transferred to lysosomes. VTA1 interacts with charged multivesicular body protein 5 (CHMP5) to form stable complexes in the cytoplasm, binds well to CHMP1B, CHMP2A, and CHMP3 proteins for MVB sorting, and is required for immunodeficiency virus type 1 HIV particle release.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells and incubate for 2 hours

Wash wells, add 50 µL Biotinylated Antibody to each well and incubate for 1 hours

Wash wells, add 50 µL Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes

Wash wells, add 50 µL Chromogen Substrate to each well and incubate for 15 minutes

Add 50 µL Stop Solution to each well and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store kit at 4°C immediately upon receipt, apart from Biotinylated Human VTA1 and 100X Streptavidin-Peroxidase Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human VTA1 coated Microplate (12 x 8 wells)	96 wells	+4°C
Human VTA1 Standard (Lyophilized)	1 vial	+4°C
Biotinylated Human VTA1	120 µL	-20°C
10X Diluent M Concentrate	20 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
Chromogen Substrate	7 mL	+4°C
Stop Solution	11 mL	+4°C
Sealing Tapes	3 units	+4°C
1X Standard Diluent	2 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform 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.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 10X Diluent M Concentrate:

Prepare 1X Diluent M by diluting Diluent M Concentrate 1 in 10 with reagent grade water. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at +4°C.

9.2 20X Wash Buffer Concentrate:

Prepare 1X Wash Buffer by diluting Wash Buffer Concentrate 1 in 20 with reagent grade water. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

9.3 Biotinylated Human VTA1:

Spin down the antibody briefly and dilute the desired amount of the antibody 1 in 50 with 1X Diluent M. Any remaining solution should be frozen at -20°C.

9.4 100X Streptavidin-Peroxidase Conjugate:

Spin down the Streptavidin-Peroxidase Conjugate briefly and dilute the desired amount of the conjugate 1 in 100 with 1X Diluent M. Any remaining solution should be frozen at -20°C.

9.5 Anti-Human VTA1 coated Microplate (12 x 8 wells):

Ready to use. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

9.6 Chromogen Substrate (8 mL):

Ready to use. Store at +4°C.

9.7 Sealing Tapes (3 units):

Ready to use. Store at +4°C.

9.8 Stop Solution (12 mL):

Ready to use. Store at +4°C.

9.9 1X Standard Diluent (2 mL)

Ready to use. Store at +4°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the VTA1 Stock to generate a 24 ng/mL **Stock Standard #1**.

10.1.1 First consult the VTA1 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Standard Diluent to add when resuspending the VTA1 Standard vial to produce a 24 ng/mL VTA1 Standard stock by using the following equation:

CS = Starting mass of VTA1 Standard stock (see vial label) (ng)

CF = 24 ng/mL VTA1 Standard #1 final required concentration

VD = Required volume of 1X Standard Diluent for reconstitution (µL)

Calculate total required volume 1X Standard Diluent for resuspension:

$$(C_S / C_S) * 1{,}000 = V_D$$

Example:

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

C_S = 12 ng of VTA1 Standard in vial

C_F = 24 ng/mL VTA1 Standard **#1** final concentration

V_D = Required volume of 1X Standard Diluent for reconstitution

(12 ng / 24 ng/mL) * 1,000 = 500 µL

10.1.3 First briefly centrifuge the VTA1 Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the VTA1 Standard vial by adding the appropriate calculated amount VD of 1X Standard Diluent to the vial to generate the 24 ng/mL VTA1 **Stock Standard.** Mix gently and thoroughly.

10.2 Allow the reconstituted 24 ng/mL VTA1 **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label eight tubes #1 – 8.

10.4 Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (24 ng/ml) 1 in 2 with 1X Diluent M to produce 12.0, 6.0, 3.0, 1.5, 0.75, 0.375 and 0.188 ng/ml solutions. 1X Diluent M serves as the zero standard (0 ng/ml).

10.5 Add 120 µL of 1X Diluent M to tube #2 – 8.

10.6 To prepare **Standard #1**, add 120 µL of the **Stock Standard** into tube #1 and mix gently.

10.7 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

Standard #	Volume to dilute (µL)	Volume Diluent M (µL)	VTA1 (ng/mL)
1	Step 10.6		12
2	120 µL Standard #1	120	6.0
3	120 µL Standard #2	120	3.0
4	120 µL Standard #3	120	1.5
5	120 µL Standard #4	120	0.75
6	120 µL Standard #5	120	0.375
7	120 µL Standard #6	120	0.188
8 (Blank)	N/A	120	0

11. Sample Preparation

11.1 Cell Lysate:

Rinse cells with cold PBS and then scrape the cells into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend the pellet in ice-cold Lysis Buffer (10 mM Tris, pH8.0, 130 mM NaCl, 1% Triton X-100, protease inhibitor cocktail). For every 1 x 10^6 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. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.2 Cell Culture Supernatants:

Centrifuge cell culture media at 1500 rpm for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -80°C. Avoid repeated freeze-thaw cycles.

12. 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.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and store in a vacuum desiccator.

12.3 Add 50 µL of all sample or standard to appropriate wells. Gently tap plate to thoroughly coat the wells. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

12.4 Wash five times with 200 µl of Wash Buffer manually. Wash by aspirating or decanting from wells then dispensing 200 µL Wash Buffer into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid. If using a machine, wash six times with 300 µl of Wash Buffer.

12.5 Add 50 µl of 1X Biotinylated Human VTA1 to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed and incubate for 1 hour.

12.6 Wash the microplate as described above (Step 12.4).

12.7 Add 50 µl of 1X Streptavidin-Peroxidase 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.

12.8 Turn on the microplate reader and set up the program in advance.

12.9 Wash the microplate as described above (Step 12.4).

12.10 Add 50 µl of Chromogen Substrate per well and incubate for 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.

12.11 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.

12.12 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.

∆ **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.

13. Calculations

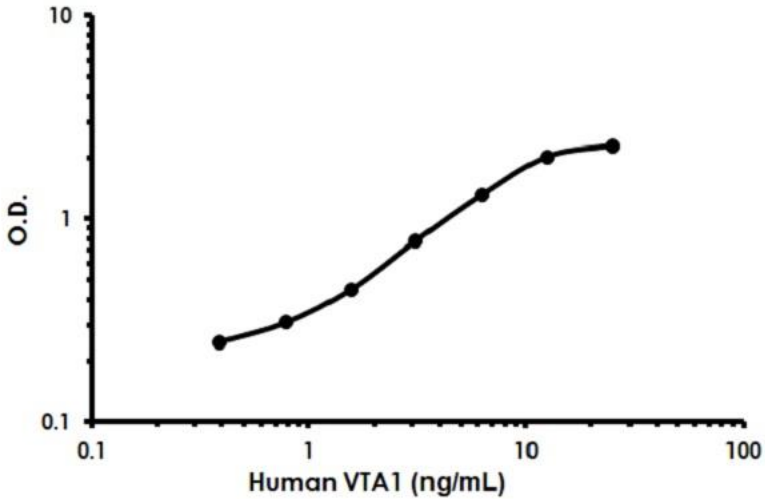
13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.

13.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.

13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements	
Concentration (ng/mL)	**Mean O.D**
0	0.121
0.391	0.248
0.781	0.308
1.563	0.450
3.125	0.781
6.250	1.310
12.5	2.022
25	2.282

Figure 1. Example of human VTA1 standard curve in 1X Diluent M. The VTA1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

15. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 60 pg/ml. The MDD was determined by calculating the mean of zero standard and adding 2 standard deviations then extrapolating the corresponding concentration.

PRECISION –

Intra-assay precision was determined by testing three

reference control samples twenty times in one assay.

Inter-assay precision was determined by testing three reference control samples in twenty assays.

	Intra-Assay Precision			**Inter-Assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	4.8%	4.4%	3.9%	10.7%	10.5%	9.6%
Average CV (%)	4.4%			10.3%		

RECOVERY –

Standard Added Value	0.75-6 ng/ml
Recovery (%)	91 – 105 %
Average Recovery (%)	97%

16. Assay Specificity

This kit recognizes human VTA1 protein in cell lysate and cell culture supernatant.

17. Species Reactivity

This assay recognizes both natural and recombinant human VTA1 protein.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.

	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)