Quantikine® ELISA

Human ADAMTS13 Immunoassay

Catalog Number DADT130

For the quantitative determination of human A Disintegrin And Metalloproteinase with Thombospondin type 1 motif, 13 (ADAMTS13) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

ADAMTS13 (A Disintegrin And Metalloproteinase with Thrombospondin type 1 motif, 13), also known as von Willebrand factor (vWF)-cleaving protease, belongs to the ADAMTS superfamily of secreted multidomain zinc metalloproteases. Full-length human ADAMTS13 contains 1427 amino acid residues that are organized into the following domains: signal peptide, propeptide, catalytic, disintegrin-like, thrombospondin type 1 (TSP1), cysteine rich, spacer, seven TSP1 repeats, and two CUB domains. The propeptide contains a furin cleavage consensus motif and is most likely removed in the Golgi or at the cell surface, releasing the 190 kDa active enzyme (1-4). ADAMTS13 is mainly produced by the liver and circulates in the blood (5).

vWF, the only known substrate for ADAMTS13, is a large multimeric glycoprotein that is required for platelet adhesion and thrombus formation (1, 2, 6). The hemostatic potential of vWF increases with its length. ADAMTS13 cleaves the ultra-large multimeric vWF (ULvWF) within the A2 domain between Tyr1605 and Met1606, generating smaller, less adhesive multimers (1, 7). Shear stress is required to induce a conformational change in the ULvWF fibers to expose the cleavage site (8). In addition to its role in platelet adhesion, ULvWF has been shown to promote leukocyte adhesion and extravasation during inflammation (9). By cleaving ULvWF, ADAMTS13 down-regulates not only thrombosis but also inflammation. As a result, decreased ADAMTS13 activity accelerates inflammatory diseases and is associated with acute and chronic inflammation (9, 10).

ADAMTS13 deficiency as a result of hereditary gene mutations or inhibitory autoantibodies to ADAMTS13 is a cause of thrombotic thrombocytopenic purpura (TTP), which is a rare condition of the blood-coagulation system. TTP is characterized by extensive formation of microthrombi in the microvasculature and may be accompanied by neurological dysfunction and renal failure (11-15). Additional pathologies associated with decreased circulating ADAMTS13 include liver cirrhosis, sepsis, alcoholic hepatitis, and acute pancreatitis (16-19). Low plasma ADAMTS13 is a useful predictor of cardiac and cerebrovascular events in coronary artery diseases (20, 21). After acute myocardial infarction, early decrease of ADAMTS13 levels is a predictor of future thrombotic events (22). In atrial fibrillation (AF), ADAMTS13 level in patients after treatment with cardioconversion is useful for prediction of recurrence of AF (23).

The Quantikine® Human ADAMTS13 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human ADAMTS13 in cell culture supernates, serum, and plasma. It contains CHO cell-expressed recombinant human ADAMTS13 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ADAMTS13 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human ADAMTS13.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ADAMTS13 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ADAMTS13 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ADAMTS13 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ADAMTS13 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until these factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Human ADAMTS13 Microplate	894000	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ADAMTS13.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ADAMTS13 Conjugate	894001	21 mL of a polyclonal antibody specific for human ADAMTS13 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human ADAMTS13 Standard	894002	Recombinant human ADAMTS13 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-89	895881	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	
Color Reagent A	895000	12.5 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12.5 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human ADAMTS13 Controls (optional; R&D Systems®, Catalog # QC35).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

Plasma - Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

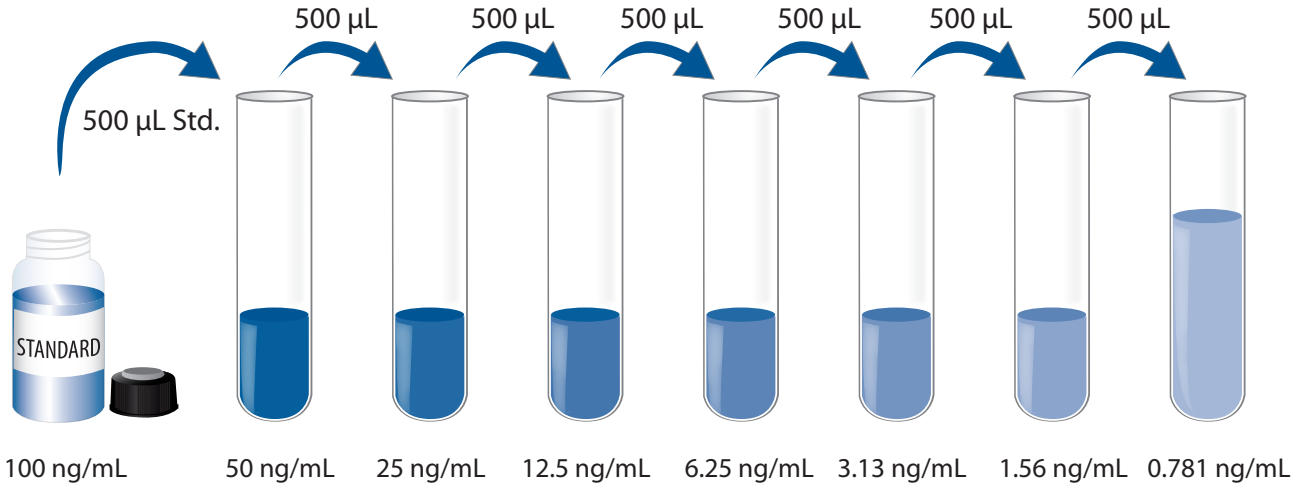
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 10 mL of Calibrator Diluent RD5-26 Concentrate to 30 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Human ADAMTS13 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ADAMTS13 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes. Mix well prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-89 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human ADAMTS13 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. **Protect from light.** Incubate for 30 minutes at room temperature.
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

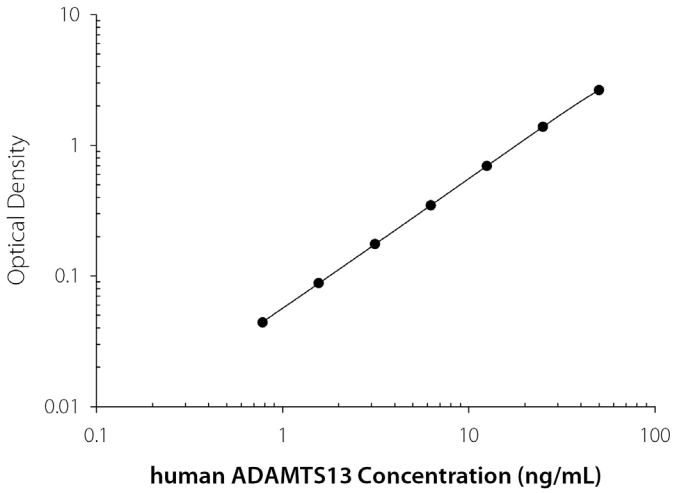
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human ADAMTS13 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.013 0.014	0.014	—
0.781	0.054 0.056	0.055	0.041
1.56	0.085 0.087	0.086	0.072
3.13	0.159 0.163	0.161	0.147
6.25	0.302 0.310	0.306	0.292
12.5	0.603 0.613	0.608	0.594
25	1.212 1.230	1.221	1.207
50	2.396 2.410	2.403	2.389

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	4.78	13.8	29.7	4.51	14.2	30.2
Standard deviation	0.177	0.364	0.685	0.256	0.722	1.08
CV (%)	3.7	2.6	2.3	5.7	5.1	3.6

RECOVERY

The recovery of human ADAMTS13 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	100	91-111%
Serum* (n=4)	97	85-106%
EDTA plasma* (n=4)	97	89-109%
Heparin plasma* (n=4)	97	85-115%
Citrate plasma* (n=4)	97	87-114%

*Samples were diluted as directed in Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ADAMTS13 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Citrate plasma* (n=4)
1:2	Average % of Expected	100	99	100	99	100
	Range (%)	98-102	98-100	99-100	92-104	98-102
1:4	Average % of Expected	99	104	106	102	103
	Range (%)	96-103	102-106	106-106	99-104	100-104
1:8	Average % of Expected	100	104	110	107	103
	Range (%)	92-108	103-106	108-111	101-112	99-105
1:16	Average % of Expected	93	105	106	108	102
	Range (%)	85-102	99-108	98-113	100-112	98-110

* Samples were diluted 50-fold prior to assay.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human ADAMTS13 ranged from 0.010-0.260 ng/mL. The mean MDD was 0.104 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay in calibrated against a highly purified CHO cell-expressed recombinant human ADAMTS13 (amino acids Gln34-Thr1427; Accession # Q76LX8).

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human ADAMTS13 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	985	515-1644	207
EDTA plasma (n=35)	758	401-1271	166
Heparin plasma (n=35)	915	472-1729	216
Citrate plasma (n=35)	797	370-1403	179

Cell Culture Supernates - Aliquots of cell culture supernates from 16 various cell lines were assayed for levels of human ADAMTS13. All samples measured below the lowest standard, 0.781 ng/mL.

SPECIFICITY

This assay recognizes recombinant and natural human ADAMTS13. The factors listed below were prepared at 500 ng/mL in calibrator diluent and assayed for cross-reactivity.

Preparations of the following factors at 500 ng/mL in a mid-range recombinant human ADAMTS13 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ADAMTS1
ADAMTS4
ADAMTS5
ADAMTS10
ADAMTS12
ADAMTS15
TIMP-3
vWF-A2

Natural proteins:

human von Willebrand Factor (vWF)

This immunoassay also recognizes a truncated version of recombinant human ADAMTS13 (amino acids Gln34-Trp688; Accession # NP_620594).

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