Quantikine® ELISA

Human ADAM12 Immunoassay

Catalog Number DAD120

For the quantitative determination of A Disintegrin And Metalloproteinase domain-containing protein 12 (ADAM12) concentrations in cell culture supernates, serum, plasma, and urine.

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

Human A Disintegrin And Metalloproteinase domain-containing protein 12 (ADAM12) belongs to the ADAM metalloproteinase family. It is a single polypeptide chain composed of multiple domains, including the signal peptide, propeptide, metalloproteinase, disintegrin, cystein-rich, and EGF-like domains, as well as the transmembrane segment and cytoplasmic tail. Human ADAM12 has two alternatively transcribed forms: a membrane-anchored long form of 110 kDa and a 90 kDa shorter secreted form lacking the transmembrane domain and the cytoplasmic tail (1-3). ADAM12 is initially synthesized as an inactive enzyme. Its latency requires the coordination of a cysteine residue in the pro-domain with the Zn^{2+} in the active site of the catalytic domain. It is then activated by a furin-like protease in the trans-Golgi apparatus through cleavage of the pro-domain before it is transported to the cell surface and secreted as the active form (4, 5). Upon activation, the pro-domain remains non-covalently attached to the mature enzyme (6). Endogenous inhibitors for ADAM12 include TIMP-3 and α_2-macroglobulin (7). The soluble form of ADAM12 is detectable in various body fluids, such as serum and urine (8-10).

Like other metalloproteinases, ADAM12 possesses gelatinase activity. It can cleave extracellular matrix proteins, such as gelatin, type IV collagen, and fibronectin (10). It may play important roles in cell adhesion and extracellular matrix degradation. Over-expression of ADAM12 has been observed in various types of cancer. In mouse prostate cancer, ADAM12 is highly expressed in the carcinoma-associated stroma, and it is required for tumor progression (11). In a mouse breast cancer model, ADAM12 accelerates tumor progression by increasing the apoptotic sensitivity of normal stromal cells while rendering tumor cells more resistant to apoptosis (12, 13). Additionally, it has been reported that in breast cancer patients, ADAM12 is significantly increased in the urine compared to healthy controls, and higher levels of urinary ADAM12 seem to correlate with more aggressive phenotypes and more advanced stages (10).

ADAM12 may also participate in cell signaling. It has been shown that it can facilitate the phosphorylation of Smad2 and the stabilization of the TGF-β receptor type II (14). It is likely that ADAM12 contributes to TGF-β signaling via a mechanism independent of its protease activity. Additionally, the cytoplasmic tail of ADAM12 can directly interact with a number of cell signaling molecules, such as α-actinin, c-Src, and PI-3 kinase (15-17). Substrates for ADAM12 also include IGFBP-3 and IGFBP-5 (8). ADAM12 is abundantly expressed in the placenta. During pregnancy, serum levels steadily increase, which correlates with the elevation of IGFBP-3 proteolysis. Thus, it has been postulated that ADAM12 may be the enzyme that is responsible for the cleavage of IGFBP-3 during pregnancy (8). Abnormal ADAM12 serum levels in pregnant women are associated with a number of neonatal and pregnancy-related disorders, such as Down's syndrome, chromosome 18 trisomy, intra-uterine fetal growth restriction, and preeclampsia (9, 18-21).

The Quantikine Human ADAM12 immunoassay is a 4.5 hour solid phase ELISA designed to measure human ADAM12 in cell culture supernates, serum, plasma, and urine. It contains CHO cell-expressed recombinant human ADAM12 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural ADAM12 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 naturally occurring human ADAM12.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ADAM12 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ADAM12 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human ADAM12 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 ADAM12 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, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all 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 ADAM12 Microplate	893923	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ADAM12.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ADAM12 Standard	893925	2 vials of recombinant human ADAM12 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Human ADAM12 Conjugate	893924	21 mL of a monoclonal antibody specific for human ADAM12 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives. *May contain crystals. Warm to room temperature and mix well to dissolve.*	
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 preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 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.
- 50 mL and 500 mL graduated cylinders.
- 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 ADAM12 Controls (optional; available from R&D Systems).

PRECAUTIONS

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

Some components in this kit contain ProClin® 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. Please refer to the MSDS 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 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.

Note: *EDTA plasma is not suitable for use in this assay due to its chelating properties. Citrate plasma has not been validated for use in this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum samples from pregnant donors require at least a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

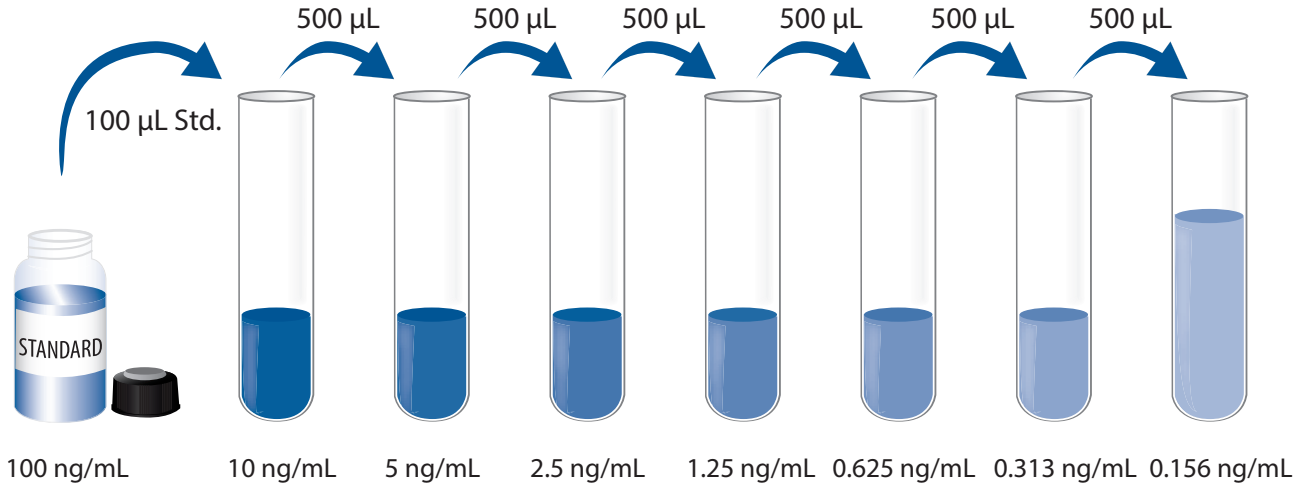
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 12.5 mL of Calibrator Diluent RD5-26 Concentrate to 37.5 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Human ADAM12 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ADAM12 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 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 10 ng/mL tube. Pipette 500 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 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 samples, controls, and standards 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 RD1X to each well. *Assay Diluent RD1X may contain crystals. Warm to room temperature and mix well to dissolve before use.*
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 ADAM12 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. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
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 the Sample Preparation section.

CALCULATION OF RESULTS

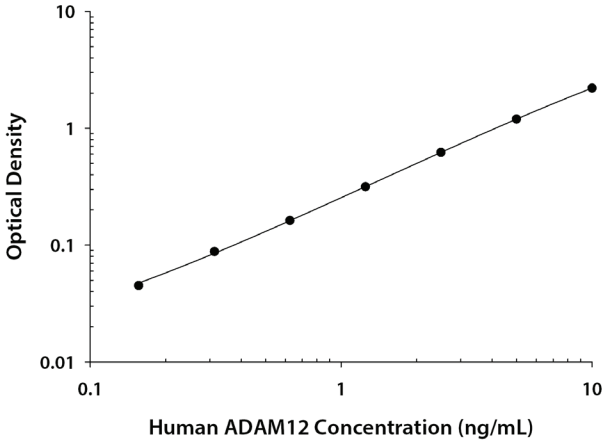
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 ADAM12 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	0.034 0.042	0.038	—
0.156	0.082 0.083	0.083	0.045
0.313	0.122 0.129	0.126	0.088
0.625	0.198 0.202	0.200	0.162
1.25	0.354 0.354	0.354	0.316
2.5	0.646 0.669	0.658	0.620
5	1.204 1.267	1.236	1.198
10	2.242 2.244	2.243	2.205

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)	1.39	3.57	6.84	1.43	3.62	6.74
Standard deviation	0.057	0.131	0.235	0.079	0.145	0.299
CV (%)	4.1	3.7	3.4	5.5	4.0	4.4

RECOVERY

The recovery of human ADAM12 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	98	88-105%
Serum (n=4)	98	89-106%
Heparin plasma (n=4)	97	92-106%
Urine (n=4)	99	91-106%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ADAM12 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Serum (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	100	107	100	101
	Range (%)	95-105	105-108	97-106	96-104
1:4	Average % of Expected	102	108	100	102
	Range (%)	96-107	103-112	93-109	94-108
1:8	Average % of Expected	100	108	100	101
	Range (%)	89-108	105-109	93-110	92-110
1:16	Average % of Expected	99	98	100	101
	Range (%)	87-115	89-106	88-115	91-109

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human ADAM12 ranged from 0.016-0.076 ng/mL. The mean MDD was 0.030 ng/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human ADAM12 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human ADAM12 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Serum (n=35)	0.309	20	ND-0.473
Heparin plasma (n=35)	0.303	20	ND-0.506
Urine (n=11)	3.59	91	ND-15.2

ND=Non-detectable

Cell Culture Supernates - U-87 MG human glioblastoma/astrocytoma cells were cultured in MEM media supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 1 mM sodium pyruvate, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for natural human ADAM12, and measured 0.164 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human pro-, mature, and TIMP-3 complexed ADAM12.

The factors listed below were prepared at 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human ADAM12 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ADAM8
ADAM9
ADAM10
ADAM15
ADAM19
ADAM22
ADAM23
ADAM33
ADAMTS1
ADAMTS4
ADAMTS5
ADAMTS13
ADAMTS15
IGFBP-3
IGFBP-5
MMP-2
MMP-9
TACE/ADAM17
TIMP-1
TIMP-2
TIMP-3
TIMP-4

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