KAMIYA BIOMEDICAL COMPANY

ADAMTS5 Assay

For the quantitative determination of human ADAMTS5 mRNA in whole blood, plasma, urine and transplantation tissue

Cat. No. KT-697

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** ADAMTS5 Assay is a real time PCR assay for the quantitative determination of human ADAMTS5 (a disintegrin and metalloproteinase with thrombospondin motifs 5) mRNA in whole blood, plasma, urine and transplantation tissue. For research use only. Not for use in diagnostic procedures.

INTRODUCTION
Aggrecan is the major proteoglycan in cartilage, and is degraded by ADAMTS5 in arthritic cartilage. Studies in models of cultured bovine and porcine chondrocytes and cartilage explants have reported that ADAMTS5 is not induced by stimulation with IL-1 and TNFα. It has also been shown that ADAMTS5 expression in human synoviocytes is not inhibited by the anti-TNF biologics.

PRINCIPLE
ADAMTS5 TEST Kit contains specific primers, probes and calibrator DNA for the detection and quantification of human ADAMTS5 mRNA in samples. The test is based on real-time PCR that, in addition to specific forward and reverse oligonucleotide primers, utilizes MGB (minor groove binder) probe to generate a fluorescent signal when specific cDNA is present in samples. Samples, such as whole blood, plasma, urine, and transplantation tissue can be used.

COMPONENTS
Master Mix: 1 vial, 900 µL
ADAMTS5 MGB Probe: 1 vial, 45 µL
β-actin MGB Probe: 1 vial, 45 µL
ADAMTS5 calibrator DNA: 1 vial, 50 µL
β-actin calibrator DNA: 1 vial, 50 µL

Each kit contains enough reagents to perform 21 tests. Each kit also contains a package insert.

Materials or Equipment required but not provided
- Thermal Cycler for real-time PCR [ABI 7500 Real-Time PCR Systems (Life Technologies™) is recommended.]
- Pipets (0.5 µL - 1 mL) with sterile filter tips
- Sterile microtubes
- Nuclease-free water

WARNINGS AND PRECAUTIONS
- This assay must be carried out by skilled personnel.
- Samples should be regarded as potentially infectious materials.

PROCEDURE
The complete procedure is separated into five steps:

1. Sample preparation and RNA extraction
2. Preparation of cDNA
3. Preparation of calibrator DNA
4. ADAMTS5 Real-time PCR Protocol
5. Calculation of ADAMTS5 mRNA

1. SAMPLE COLLECTION AND RNA EXTRACTION

1.1 Collect 2.5 – 5.0 mLof peripheral blood using an EDTA-2Na+ tube or PAXgene Blood RNA Tube® (PreAnalytiX).
1.2 Extract total RNA by use of a commercial RNA extraction kit from samples according to the manufacturer's instructions within 72 hours.
1.3 Dilute total RNA to 1.0 µg/10 µL with nuclease-free water.

2. PREPARATION OF cDNA

Purify cDNA from 1.0 µg of total RNA prepared as Section 1.3 using a commercial RT kit [RETROscript® (Ambion) with 2-step RT-PCR is recommended to purify cDNA and adjust the final reaction volume to 20 µL].

3. PREPARATION OF CALIBRATOR DNA

Prepare calibrator DNA (Calibrator 2 through Calibrator 6) for ADAMTS5 or β-actin by diluting ADAMTS5 calibrator DNA (= Calibrator 1) or β-actin calibrator DNA (= Calibrator 1).

	Dilution	
Standard 1 (1.0×10^3 ng/mL)	–	–
Standard 2 (1.0×10^2 ng/mL)	Standard 1 20µl	Nuclease free water 180µl
Standard 3 (1.0×10^1 ng/mL)	Standard 2 20µl	Nuclease free water 180µl
Standard 4 (1.0×10^0 ng/mL)	Standard 3 20µl	Nuclease free water 180µl
Standard 5 (1.0×10^{-1} ng/mL)	Standard 4 20µl	Nuclease free water 180µl
Standard 6 (1.0×10^{-2} ng/mL)	Standard 5 20µl	Nuclease free water 180µl
Standard 7 (1.0×10^{-3} ng/mL)	Standard 6 20µl	Nuclease free water 180µl

4. ADAMTS5 Real-time PCR Protocol

Please read carefully the manufacturer's instructions before starting the procedure. Always use filter tips for pipetting.

4.1 Mix gently (do NOT vortex) the following reagents in a sterile tube:
Master Mix, MGB Probe, Template DNA, Nuclease-free water. Spin down briefly. Cool all reagents during all work steps.

PCR Reaction Volume	
Master Mix	15 µl
MGB Probe*1)	1.5 µl
Template DNA *2)	3.0 µl
Nuclease-free water	10.5 µl
Total	30 µl

*1) MGB Probe contains both FAM/MGB probe and primers.
*2) In case of ADAMTS5, 2 fold dilution of the cDNA prepared as Section 2. In case of β-actin, 50 fold dilution of the cDNA prepared as Section 2.

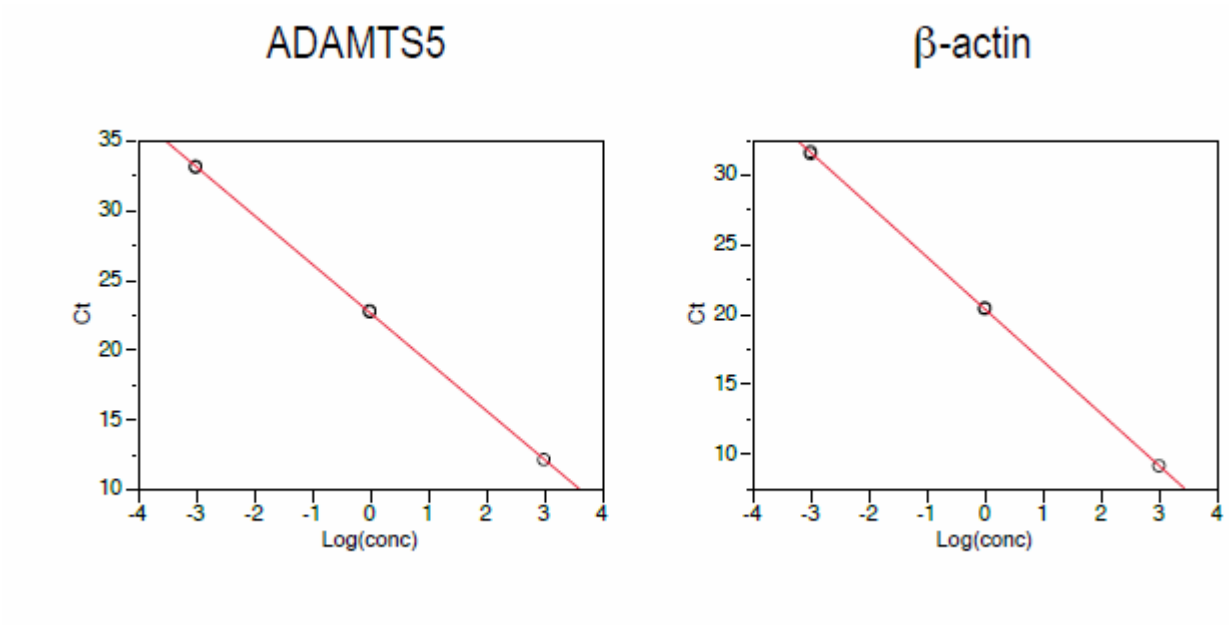
4.2 Run the thermal cycler using the following temperature protocol:
50℃ for 2 min
95℃ for 10 min
50 cycles of:
95℃ for 15 sec
60℃ for 1 min

5. CALCULATION OF ADAMTS5 mRNA

5.1 Preparation of the calibration curve
A vertical axis is set as the Ct value provided as a result of the real time PCR performed by duplicate using Calibrator1, Calibrator4, and Calibrator7 of template DNA. A horizontal axis is set as the logarithms (Log10 (Conc)) of the concentration (Conc) (ng/mL) of the template DNA.

5.2 Calculation of ADAMTS5 and β-actin mRNA
Following is an example for the calibration curve making

MGB Probe	Template DNA, Conc(ng/mL)	Log_{10}(Conc)	Ct
ADAMTS5	Standard 1, 1.0×10^3	3	12.07
ADAMTS5	Standard 1, 1.0×10^3	3	12.09
ADAMTS5	Standard 4, 1.0	0	22.69
ADAMTS5	Standard 4, 1.0	0	22.77
ADAMTS5	Standard 7, 1.0×10^{-3}	-3	33.07
ADAMTS5	Standard 7, 1.0×10^{-3}	-3	33.14
β-actin	Standard 1, 1.0×10^3	3	9.1
β-actin	Standard 1, 1.0×10^3	3	9.11
β-actin	Standard 4, 1.0	0	20.36
β-actin	Standard 4, 1.0	0	20.43
β-actin	Standard 7, 1.0×10^{-3}	-3	31.63
β-actin	Standard 7, 1.0×10^{-3}	-3	31.48



Calculated calibration curve should be:

ADAMTS5: Ct = 22.64 – 3.504 × Log_{10} (Conc) (^{r2}=0.99993)

β-actin: Ct = 20.35 – 3.741 × Log_{10} (Conc) (^{r2}=0.99996)

The amount of ADAMTS5 mRNA is calculated as the ratio with the quantity of β-actin mRNA.
For example, when the Ct is 34.24 (ADAMTS5) and 23.55 (β-actin);
Conc (ADAMTS5) =10^ [(22.64 – 34.24)/ 3.504]
Conc(β-actin) =10^ [(20.35 – 23.55)/ 3.741]
Therefore, Amount of ADAMTS5 mRNA
= Conc (ADAMTS5) x dilution ratio / Conc (β-actin) x dilution ratio
= 10^ [(22.64 – 34.24)/ 3.504] × 2 /10^ [(20.35 – 23.55)/ 3.741] × 50
= 1.402 × 10-4

STORAGE

All reagents should be stored at -20℃. All reagents can be used until the expiry date printed on the labels.
Avoid more than 2 freezing and thawing cycles of the reagents. Cool all reagents during work steps.

FOR RESEARCH USE ONLY

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