

## ADAMTS5 TEST Kit

For research use only

Real-time PCR assay for the quantification of ADAMTS5 (a disintegrin and metalloproteinase with thrombospondin motifs 5) mRNA.

**Cat. No: KTB36450**

**For research use 21 Determinations**

### INTENDED USE

ADAMTS5 TEST Kit is a quantitative assay for ADAMTS5 (a disintegrin and metalloproteinase with thrombospondin motifs 5) mRNA in clinical specimens (whole blood, plasma, urine, transplantation tissue, etc.) using real-time PCR system.

Ref.	Type of Reagent	Presentation	Cap Color
<b>KTB36451</b>	Master Mix	1 vial,900 µl	white
<b>KTB36452</b>	ADAMTS5 MGB Probe	1 vial,45 µl	red
<b>KTB36453</b>	β-actin MGB Probe	1 vial,45 µl	purple
<b>KTB36454</b>	ADAMTS5 standard DNA	1 vial,50 µl	yellow
<b>KTB36455</b>	β-actin standard DNA	1 vial,50 µl	green

### SUMMARY

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 [1,2]. It has also been shown that ADAMTS5 expression in human synoviocytes is not inhibited by the anti-TNF biologics.

### PRINCIPLE OF THE TEST

ADAMTS5 TEST Kit contains specific primers, probes and standard DNA for the detection and quantification of human ADAMTS5 mRNA in clinical 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. Clinical samples, such as whole blood, plasma, urine, and transplantation tissue can be used.

### REAGENTS PROVIDED

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

### STORAGE AND HANDLING

All reagents (KTB36451 to KTB36455) should be stored at -20°C. 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.

### REQUIRED MATERIAL NOT PROVIDED

1. Thermal Cycler for real-time PCR [ABI 7500 Real-Time PCR Systems (Life Technologies™) is recommended.]
2. Pipets (0.5µl - 1 ml) with sterile filter tips
3. Sterile microtubes
4. Nuclease-free water

### WARNINGS AND PRECAUTIONS

- This assay must be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious

materials.

### ASSAY PROCEDURE

The complete procedure is separated into five steps:

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

#### 1. SAMPLE COLLECTION AND RNA EXTRACTION

- 1.1 Collect 2.5 – 5.0ml of peripheral blood using an EDTA-2Na<sup>+</sup> 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 1.0µg/10µl with nuclease-free water.

#### 2. PREPARATION OF cDNA

Purify cDNA from 1.0µg 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 20µl].

#### 3. PREPARATION OF STANDARD DNA

Prepare standard DNA (Standard 2 through Standard 6) for ADAMTS5 or β-actin by diluting ADAMTS5 standard DNA (KTB36454)(= Standard 1) or β-actin standard DNA (KTB36455) (= Standard 1).

	Dilution	
Standard 1 (1.0 × 10 <sup>3</sup> ng/mL)	-	-
Standard 2 (1.0 × 10 <sup>2</sup> ng/mL)	Standard 1 20µl	Nuclease free water 180µl
Standard 3 (1.0 × 10 <sup>1</sup> ng/mL)	Standard 2 20µl	Nuclease free water 180µl
Standard 4 (1.0 × 10 <sup>0</sup> ng/mL)	Standard 3 20µl	Nuclease free water 180µl
Standard 5 (1.0 × 10 <sup>-1</sup> ng/mL)	Standard 4 20µl	Nuclease free water 180µl
Standard 6 (1.0 × 10 <sup>-2</sup> ng/mL)	Standard 5 20µl	Nuclease free water 180µl
Standard 7 (1.0 × 10 <sup>-3</sup> 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
<b>Total</b>	<b>30 µl</b>

- \*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  $\beta$ -actin, 50 fold dilution of the cDNA prepared as Section 2.

4.2 Run the thermal cycler using the following temperature protocol:

50°C for 2 min

95°C for 10 min

50 cycles of:

{ 95°C for 15 sec  
 60°C for 1 min

## 5. CALCULATION OF ADAMTS5 mRNA

### 5.1 Preparation of the standard curve

A vertical axis is set as the Ct value provided as a result of the real time PCR performed by duplicate using Standard1, Standard4, and Standard7 of template DNA. A horizontal axis is set as the logarithms ( $\text{Log}_{10}(\text{Conc})$ ) of the concentration (Conc) (ng/mL) of the template DNA.

### 5.2 Calculation of ADAMTS5 and $\beta$ -actin mRNA

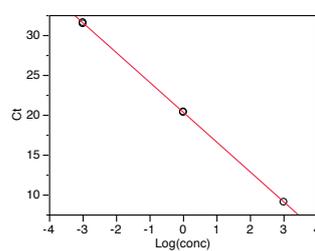
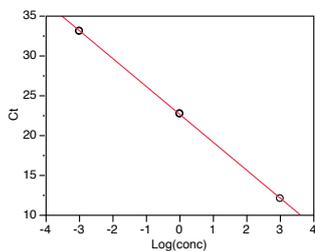
Following is an example for the standard curve making

MGB Probe	Template DNA, Conc(ng/mL)	$\text{Log}_{10}(\text{Conc})$	Ct
ADAMTS5	Standard 1, 1.0 × 10 <sup>3</sup>	3	12.07
ADAMTS5	Standard 1, 1.0 × 10 <sup>3</sup>	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 <sup>-3</sup>	-3	33.07
ADAMTS5	Standard 7, 1.0 × 10 <sup>-3</sup>	-3	33.14
$\beta$ -actin	Standard 1, 1.0 × 10 <sup>3</sup>	3	9.1
$\beta$ -actin	Standard 1, 1.0 × 10 <sup>3</sup>	3	9.11
$\beta$ -actin	Standard 4, 1.0	0	20.36
$\beta$ -actin	Standard 4, 1.0	0	20.43
$\beta$ -actin	Standard 7, 1.0 × 10 <sup>-3</sup>	-3	31.63
$\beta$ -actin	Standard 7, 1.0 × 10 <sup>-3</sup>	-3	31.48

Standard curve

ADAMTS5

$\beta$ -actin



Calculated standard curve should be:

ADAMTS5:  $\text{Ct} = 22.64 - 3.504 \times \text{Log}_{10}(\text{Conc})$   
 $(r^2=0.99993)$

$\beta$ -actin:  $\text{Ct} = 20.35 - 3.741 \times \text{Log}_{10}(\text{Conc})$   
 $(r^2=0.99996)$

The amount of ADAMTS5 mRNA is calculated as the ratio with the quantity of  $\beta$ -actin mRNA.

For example, when the Ct is 34.24 (ADAMTS5) and 23.55 ( $\beta$ -actin);

$\text{Conc}(\text{ADAMTS5}) = 10^{[(22.64 - 34.24) / 3.504]}$

$\text{Conc}(\beta\text{-actin}) = 10^{[(20.35 - 23.55) / 3.741]}$

Therefore,

Amount of ADAMTS5 mRNA

$= \text{Conc}(\text{ADAMTS5}) \times \text{dilution ratio} / \text{Conc}(\beta\text{-actin}) \times \text{dilution ratio}$

$= 10^{[(22.64 - 34.24) / 3.504]} \times 2$

$/ 10^{[(20.35 - 23.55) / 3.741]} \times 50$

$= 1.402 \times 10^{-4}$

## SIGNIFICANCE OF MEASUREMENT

Baseline low blood ADAMTS5 mRNA expression has been reported to predict the effectiveness of infliximab (IFX) in rheumatoid arthritis (RA) patients<sup>3,4</sup>.

Predictive value of the baseline low-ADAMTS5 mRNA expression ( $<1.2 \times 10^{-4}$ ) for the clinical remission (DAS28<2.6) of RA with IFX after 14 weeks

Accuracy	74.0%
Sensitivity	50.0%
Specificity	85.3%
PPV	61.5%
NPV	78.4%

Baseline high blood ADAMTS5 mRNA expression has been reported to predict the effectiveness of adalimumab (ADA) in RA patients<sup>5</sup>.

Predictive value of the baseline high-ADAMTS5 mRNA expression ( $>1.7 \times 10^{-4}$ ) for the clinical remission (DAS28<2.6) of RA with ADA after 20 weeks

Accuracy	84.2%
Sensitivity	71.4%
Specificity	86.0%
PPV	41.7%
NPV	95.6%

## REFERENCES

1. Stanton H, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature* 434: 648-652, 2005
2. Amanda J, et al. ADAMTS-5: THE STORY SO FAR. *European Cells and Materials* 15: 11-26, 2008
3. Tsuzaka K, et al. ADAMTS5 is a biomarker for prediction of the response to infliximab in patients with rheumatoid arthritis. *Arthritis Rheum* 60: S345, 2009
4. Tsuzaka K, et al. ADAMTS5 is a biomarker for prediction of the response to Infliximab in patients with rheumatoid arthritis. *J Rheumatol* 37: 1454-1460, 2010
5. Tsuzaka K, et al. Baseline ADAMTS5 expression could sort the prediction of response to infliximab or adalimumab in patients with rheumatoid arthritis. *Arthritis Rheum* 62: S724, 2010

## NOTICE TO PERCHASER

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