

## RIDASCREEN® UST Monitoring

**REF** G09049



## 1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® UST Monitoring is an enzyme linked immunosorbent assay intended for the quantitative determination of ustekinumab (UST, Stelara®) in human serum and plasma.

## 2. Summary and explanation of the test

### Therapeutic drug monitoring

Ustekinumab (UST) is a fully human monoclonal antibody that binds to the p40 subunit common to IL-12 and IL-23 thereby preventing the interaction with the cytokine receptors on T cells, natural killer cells and antigen-presenting cells.<sup>1</sup> UST has been approved for treatment of moderate to severe Crohn's disease (CD), plaque psoriasis and psoriatic arthritis.<sup>2-4</sup>

A drug can only exert its pharmacological effect when adequate concentrations are achieved in the circulation. The serum concentration of biologicals just before the next administration, defined as trough concentration, has been used for therapeutic drug monitoring (TDM). Recent data have shown a positive relationship between UST serum concentration, either measured at trough or at an intermediate time point, and clinical outcomes in patients with Crohn's disease and plaque psoriasis, respectively. TDM may therefore be very instrumental to optimize treatment.

The RIDASCREEN® UST Monitoring uses highly specific monoclonal antibodies developed at the University of Leuven, Belgium (KU Leuven). Anti-TNF drugs (like infliximab, adalimumab, golimumab) or anti-integrin  $\alpha_4\beta_7$  drugs (like vedolizumab) do not interfere with the measurement.

As an example of TDM, the use of UST concentration measurements in plaque psoriasis and CD is described.

### Crohn's Disease

UST is administered intravenously (IV) at week 0 and thereafter every 8 weeks subcutaneously (SC). The UNITI-1 and -2 induction trials demonstrated that 33.7 % and 55.5 % of patients had a clinical response at week 6, respectively. During maintenance therapy with SC ustekinumab every 8 weeks, 53.1 % of patients were in remission at week 44 in the IM-UNITI trial.<sup>2</sup>

Several studies have demonstrated the relationship between ustekinumab trough concentrations and clinical, biological and endoscopic response, indicating the usefulness of therapeutic drug monitoring to guide clinical decision-making.<sup>5-7</sup>

### Plaque psoriasis

UST is weight-based administered subcutaneously at week 0, at week 4 and thereafter every 12 weeks. The Phoenix 1 and 2 trials indicate that treatment with ustekinumab results in rapid, significant improvements in patients with moderate-to-severe psoriasis.<sup>3,4</sup>

A recent study demonstrated a concentration-response relationship at week 4 upon injection for ustekinumab-treated psoriasis patients, indicating that monitoring 4-week post injection ustekinumab concentrations could timely identify underexposed patients who might benefit from treatment optimization.<sup>8</sup>

### **Immunogenicity**

It has been shown that the immunogenicity of ustekinumab is very low.<sup>8,9</sup>

### **3. Test principle**

In RIDASCREEN® UST Monitoring, two highly specific monoclonal antibodies (MA-UST56C1H12 and MA-UST56A2D11, both isolated and characterized at KU Leuven) are used. Anti-ustekinumab monoclonal antibodies (MA-UST56C1H12) are applied to the surface of the well in the microwell plate. During this incubation step ustekinumab binds specifically to the antibodies on the plate. After washing, a second incubation step follows with MA-UST56A2D11, which is conjugated with horseradish peroxidase. In the presence of UST, a sandwich complex is formed between immobilized MA-UST56C1H12, UST and conjugated antibodies. Unattached enzyme-labelled antibodies are removed during a subsequent washing step. After addition of the substrate, the colorless solution in the microwells will turn blue in case of a positive test result. Upon addition of the stop reagent, the color changes from blue to yellow. The absorbance is proportional to the concentration of UST present in the sample.

#### 4. Reagents provided

One kit is sufficient for 96 determinations (Table 1).

**Tab. 1:** Reagents provided

Plate	96 det.	Microtiter plate, 12 microwell strips (can be divided) in the strip holder; coated with monoclonal antibody MA-UST56C1H12
Standard 1-6	1300 µl	6 Standards; concentrations of the standards 1 to 6: 0 / 2.5 / 10 / 20 / 60 / 120 ng/ml; contains 0.09 % NaN <sub>3</sub> ; ready for use
Low Control +	1300 µl	Low positive control; contains 8 ng/ml UST and 0.09 % NaN <sub>3</sub> ; ready for use
Control +	1300 µl	Positive control; contains 70 ng/ml UST and 0.09 % NaN <sub>3</sub> ; ready for use
Diluent	100 ml	Sample dilution buffer; contains 0.09 % NaN <sub>3</sub> ; ready for use; colored orange
Conjugate	12 ml	Conjugate; peroxidase conjugated monoclonal antibody MA-UST56A2D11; ready for use; colored red
Substrate	12 ml	Substrate; Hydrogen peroxide / tetramethylbenzidine (TMB); ready for use
Wash   20x	50 ml	Wash buffer (20-fold conc.); phosphate-buffered NaCl solution; contains detergent and antimicrobial agents
Stop	6 ml	Stop reagent; 0.5 M H <sub>2</sub> SO <sub>4</sub> ; ready for use
2 Plate covers		

Information on hazardous substances complies with the labeling requirement. For further details, see the safety data sheets (SDSs) at [www.r-biopharm.com](http://www.r-biopharm.com).

#### 5. Storage instructions

All reagents must be stored at 2 - 8 °C and can be used until the expiry date printed on the label. Opened components (reagents, microwell strips) should be stored at 2 - 8°C until next use and can be maintained for 2 months. The diluted wash buffer can be used for one month when stored at 2 - 8 °C. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.

The aluminum bag containing the microwell strips must be opened in such a way that the clip seal is not torn off. Any microwell strips which are not required must immediately be returned to the aluminum bag with the desiccant and stored at 2 - 8 °C

until all the strips have been used. The colorless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

## **6. Reagents required but not provided**

### **6.1 Reagents**

-Distilled or deionized water

### **6.2 Accessories**

- Precision micropipettes and standard laboratory pipettes
- Graduated cylinder (1000 ml)
- Clean glass or plastic tubes for the dilution of the samples
- Stopwatch
- Microplate washer or multichannel pipette (300 µl)
- Microplate reader (450 nm, reference filter 620 nm)
- Filter paper (laboratory towels)
- Waste container with 0.5 % hypochlorite solution
- 37 °C incubator

## **7. Warnings and precautions for the users**

For *in vitro* diagnostic use.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

Do not mix reagents or coated microtiter strips from kits with different lot numbers.

Some ingredients of the standards and positive control mixtures are derived from materials of biological origin. No known tests can guarantee that such materials are completely free from infectious agents. Therefore, calibrators and positive control mixtures, as well as patient samples and all materials coming into contact with them, should be treated as potentially infectious.

Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat or drink in areas where samples or test reagents are being used.

The Stop reagent contains 0.5 M sulfuric acid which is irritant. Avoid contact with the skin and clothing. In case of contact with eyes or skin, rinse with plenty of water and seek medical advice.

The reagents contain  $\text{NaN}_3$  as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

The substrate contains hydrogen peroxide and N-Methyl-2-pyrrolidone (>0,3 %). For further details, see the safety data sheets (SDSs) at [www.r-biopharm.com](http://www.r-biopharm.com).

## 8. Sample collection and storage

In this assay, EDTA-plasma samples, citrate plasma samples and serum samples may be used. Following collection, the serum should be separated from the clot as quickly as possible to avoid hemolysis. Transfer the serum to a clean storage tube. Samples can be stored at 2 - 8 °C for at least 3 - 4 days or at - 20 °C for at least 12 months. Repeated freezing and thawing should be avoided. Samples must be diluted in sample diluent (see 9.3.1.).

Diluted samples may be stored for at least 8 hours at room temperature.

## 9. Test procedure

### 9.1 General information

All reagents and the Microtiter plate **Plate** must be brought to room temperature (20 - 25 °C) before use. The microwell strips must not be removed from the aluminum bag until they have reached room temperature. The reagents must be thoroughly mixed immediately before use. Once opened, unused microwell strips (in sealed bags) and the reagents must be stored at 2 - 8 °C. Once used, the microwell strips must not be used again. The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking. In order to prevent cross contamination, the samples must be prevented from coming into direct contact with the kit components. The test must not be carried out in direct sunlight. While incubating, we recommend covering the microwell plate or placing a film on it to prevent evaporation losses.

For instructions on how to perform the assay with ELISA instruments, please contact R-Biopharm AG or your local distributor.

### 9.2 Preparing the wash buffer

Mix 1 part Wash buffer concentrate **Wash | 20x** with 19 parts distilled water (1:20). Pour 50 ml concentrate in a 1000 ml measuring cylinder and stock up with distilled water to 1000 ml. Reconstituted solution can be stored at least 1 month at 2 - 8 °C. At higher temperatures, the concentrated washing solution may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

### 9.3 Preparing the samples

Serum or plasma samples can be stored at 2 - 8 °C for 3 - 4 days, or at -20 °C for at least 12 months (also see chapter 8). Repeated freezing and thawing should be avoided. Samples must be diluted in sample diluent (see 9.3.1.).

Diluted samples may be stored for at least 8 hours at room temperature.

### 9.3.1 Sample dilution

#### a) Measurement of trough concentrations during therapy maintenance phase

To measure the trough concentration (drug concentration just before next dose administration) during the maintenance phase of the treatment, the samples are diluted 1:100:

10 µl of the sample is diluted in 990 µl sample dilution buffer Diluent (1:100).

100 µl of this final diluted sample is then used in the test.

If the sample is diluted 1:100, UST concentrations between 0.04 and 12 µg/ml can be determined.

#### b) Measurement of trough concentrations during therapy induction phase

To measure trough concentrations during (re)induction therapy or to measure intermediate drug concentrations, or concentrations > 12.0 µg/mL, the samples are diluted 1:200:

10 µl of the sample is diluted in 1990 µl sample dilution buffer Diluent (1:200).

100 µl of this final diluted sample is then used in the test.

If the sample is diluted 1:200, UST concentrations between 0.08 and 24 µg/ml can be determined.

### 9.4 First incubation

After placing a sufficient number of wells in the holder, add 100 µl of Standards 1 - 6 (Standard | 1 to Standard | 6), Positive control Control | +, Low positive control Low control | + and samples to the relevant wells. Although it might be advised to run calibrators, controls and samples in duplicate, reliable results are equally obtained by doing the analysis in singlicate. Then incubate the covered microtiter plate at 37 °C for 1 hour.

### 9.5 First washing

Careful washing is important in order to achieve the correct results and should therefore be carried out strictly in accordance with the instructions. The incubated substance in the wells must be emptied into a waste container containing hypochlorite solution for disinfection. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells. Then wash the plate 5 times using 300 µl diluted wash buffer each time (see 9.2). Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.

When using a microplate washer, make sure that the machine is correctly adjusted to the type of microwell plate being used. Also make sure that all of the liquid is sucked away during each washing phase. After washing for the last time, tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.

## 9.6 Second incubation

Add 100 µl conjugate **Conjugate** into each well. Then incubate the covered microtiter plate at 37 °C for 30 minutes.

## 9.7 Second washing

The incubated substance in the wells must be emptied into a waste container containing hypochlorite solution for disinfection. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells. Then wash the plate 5 times using 300 µl diluted wash buffer each time. Tap the plate upside down vigorously against absorbent paper to ensure complete removal of liquid from the microwells.

## 9.8 Third incubation

Add 100 µl substrate **Substrate** to each well. Then incubate the plate at 37 °C in the dark for 10 minutes. After this, stop the reaction by adding 50 µl stop reagent **Stop** to each well.

After mixing carefully (by lightly tapping the side of the plate) measure the absorbance at 450 nm (reference filter 620 nm) in a plate reader.

## 10. Quality control – indication of instability or expiration of reagents

For quality control purposes, each Standard 1 to Standard 6 **Standard | 1** – **Standard | 6**, Positive control **Control | +** and Low positive control **Low control | +** (each in duplicate recommended) must be used every time the test is carried out to ensure reagent stability and correct procedural.

The following specifications must be met during each run in order to be valid:

O. D. Value for Standard 1 **Standard | 1** < 0.080

O. D. Value for Standard 6 **Standard | 6** > 1.400

### a) If the dilution factor of 1:100 is used (maintenance therapy phase):

Concentration for the Low positive control **Low Control | +**:

0.8 µg/ml, range 0.55 - 1.10 µg/ml

Concentration for the Positive control **Control | +**:

7 µg/ml, range 5 - 10 µg/ml

### b) If the dilution factor of 1:200 is used (induction therapy phase):

Concentration for the Low positive control **Low Control | +**:

1.6 µg/ml, range 1.10 - 2.20 µg/ml

Concentration for the Positive control **Control | +**:

14 µg/ml, range 10 - 20 µg/ml



For calculating the UST concentration in the controls, the same multiplicity factor must be used as for the samples (see **Chapter 11. Evaluation and Interpretation**).

Concentration is then expressed in µg/ml.

Calculation example for dilution factor 1:100:

$$60 \text{ ng/ml} \times 100 \text{ (dilution factor)} = 6 \text{ µg/ml}$$

If the values differ from those required, if the substrate is turbid or has turned blue before adding to the wells, this may indicate that the reagents have expired. If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

In case of high background signal (OD Standard 1 > 0.08) the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

If the conditions are still not fulfilled after repeating the test, please contact the manufacturer or your local R-Biopharm distributor.

## **11. Evaluation and Interpretation**

For the analysis of the results RIDASOFT® Win.NET is required.

The RIDASOFT® Win.NET or an update is available on request from R-Biopharm AG or your local R-Biopharm distributor.

Any other evaluation software which provides the 4-parameter logistic-log-model can also be used as an alternative to RIDASOFT® Win.NET.

Evaluation of RIDASCREEN® UST Monitoring is achieved by standard curve that must always be processed when running the test.

In final quality control, R-Biopharm AG has determined the target values and the allowed range of concentration for the positive control and low-positive control for each kit lot under optimal test conditions.

The dilution factor must be taken into account when calculating the UST concentration in patient samples by multiplying the measured concentration by the dilution factor.

Example: The outcome of 1:100 diluted sample, obtained by interpolation from the calibration curve is 60 ng/ml. The corresponding UST concentration in the undiluted sample is then 6 µg/ml.

Example: The outcome of 1:200 diluted sample, obtained by interpolation from the calibration curve is 80 ng/ml. The corresponding UST concentration in the undiluted sample is then 16 µg/ml.

If using the RIDASOFT® Win.NET software the dilution factor is automatically applied when using the appropriate method:

For dilution 1:100 select: RIDASOFT® Win.NET method UST100.met.

For dilution 1:200 select: RIDASOFT® Win.NET method UST200.met.

The concentration is reported in µg/ml.

## 12. Limitations of the method

The RIDASCREEN® UST Monitoring test detects the free, functionally active proportion of UST and not the proportion of UST that is bound by anti-ustekinumab antibodies, because of immunogenicity.

Individual ustekinumab concentrations, measured using the RIDASCREEN® UST Monitoring, must not be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made.

## 13. Performance characteristics

### 13.1 Example of typical optical density (O.D.) values

Tab. 2: Example of typical optical density values

Standard	O.D.
1	0.009
2	0.085
3	0.306
4	0.608
5	1.637
6	2.660

## 13.2 Precision

### 13.2.1 Intra-assay precision

The intra-assay-precision was determined in a single run using 4 references in 20 replicates each. From the OD-values of these measurements UST concentrations have been determined. The mean value, standard deviation (SD) and coefficient of variation (CV) were calculated for each sample. The results are listed in Table 3 below.

**Tab. 3:** Intra-assay precision

Reference	1	2	3	4
Mean (µg/ml)	0.61	1.65	3.07	8.37
SD	0.04	0.08	0.18	0.76
<b>% CV</b>	<b>5.9</b>	<b>5.0</b>	<b>6.0</b>	<b>9.0</b>

### 13.2.2 Inter-assay precision

The inter-assay-precision was determined in 5 runs using 4 references in 20 replicates each. From the OD-values of these measurements UST concentrations have been determined. The mean value, standard deviation (SD) and coefficient of variation (CV) were calculated for each sample. The results are listed in Table 4 below.

**Tab. 4:** Inter-assay precision

Reference	1	2	3	4
Mean (µg/ml)	0.58	1.62	3.01	8.65
SD	0.03	0.08	0.15	0.73
<b>% CV</b>	<b>4.4</b>	<b>4.9</b>	<b>4.8</b>	<b>8.4</b>

## 13.3 Specificity

### 13.3.1 Normal human serum/plasma

Specificity was determined by testing 100 healthy donor samples from Dutch and Belgian origin. None of the samples showed a detectable concentration of UST, resulting in a specificity of 100 %.

### 13.3.2 Interference

A panel of 25 potentially interfering samples was tested consisting of HAMA positive, lipemic, high cholesterol, hemolyzed and first semester pregnant women samples. No interaction with the investigated factors was observed.

A panel of 27 rheumatoid factor positive clinical samples was tested and only in one sample a detectable level of 0,12 µg/ml was measured. Thus, rheumatoid factor might

interfere in the RIDASCREEN® UST Monitoring, but only to a small extent not exceeding the lower limit of quantification of the assay.

### 13.3.3 Cross-reactivity

No cross-reactivity has been observed for following biopharmaceuticals applied for treating auto-immune diseases: infliximab, adalimumab, golimumab and vedolizumab.

### 13.4 Analytical sensitivity

The detection limit was determined as 0.12 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.01 µg/ml.

### 13.5 Recovery

15 UST-negative samples were spiked with varying UST concentrations. Based on the OD values of this measurement, the UST concentration was determined using the standard curve and the recovery calculated. The mean recovery is 98 % (Table 5).

**Tab. 5:** Recovery of 15 UST-negative samples spiked with varying UST concentrations

<b>Reference value</b>			
<b>Matrix</b>	<b>Nr.</b>	<b>UST (µg/ml)</b>	<b>Recovery (%)</b>
<b>Reference value 0.79 µg/ml</b>			
Serum	1	0.72	90 %
	2	0.78	98 %
	3	0.82	104 %
	4	0.74	94 %
	5	0.70	89 %
EDTA Plasma	6	0.71	89 %
	7	0.82	104 %
	8	0.76	96 %
	9	0.74	94 %
	10	0.74	93 %
Citrat Plasma	11	0.79	99 %
	12	0.81	102 %
	13	0,70	88 %
	14	0.73	92 %
	15	0.74	94 %
<b>Matrix</b>	<b>Nr.</b>	<b>UST (µg/ml)</b>	<b>Recovery (%)</b>
<b>Reference value 3.26 µg/ml</b>			
Serum	1	3.34	102 %
	2	3.43	105 %
	3	3.21	99 %
	4	2.95	90 %
	5	3.23	99 %

EDTA Plasma	6	3.42	105 %
	7	3.31	102 %
	8	3.20	98 %
	9	3.34	102 %
	10	3.39	104 %
Citrat Plasma	11	3.11	95 %
	12	2.97	91 %
	13	3.14	96 %
	14	3.27	100 %
	15	3.20	98 %
Matrix	Nr.	UST (µg/ml)	Recovery (%)
<b>Reference value 8.99 µg/ml</b>			
Serum	1	8.77	98 %
	2	10.00	111 %
	3	9.33	104 %
	4	8.78	98 %
	5	8.07	90 %
EDTA Plasma	6	9.63	107 %
	7	8.97	100 %
	8*	9.49	106 %
	9	8.50	95 %
	10	9.32	104 %
	11	9.37	104 %
	12	8.75	97 %
	13	8.15	91 %
	14	8.95	100 %
	15	8.90	99 %
<b>Mean</b>			<b>98 %</b>

### 13.6 Correlation with reference assay and diagnostic sensitivity

A clinical sample panel of 15 specimens was analyzed using the RIDASCREEN® UST Monitoring and the results were compared with those of the reference assay (UST ELISA developed at KU Leuven). A correlation coefficient of 0.97 was determined.

All samples having measurable UST levels according to the reference assay were detected positive (14 specimens) resulting in a diagnostic sensitivity of 100 %.










## 14. Version history

**Tab. 6:** Version history


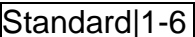
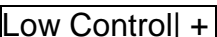
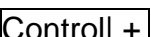
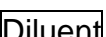
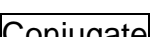
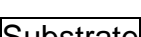
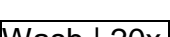

Version number	Chapter and description
2020-07-07	Release document

## 15. Explanation of symbols

### General symbols

	For <i>in vitro</i> diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

### Test specific symbols

	Microtiter plate
	Standard 1 - 6
	Low positive control
	Positive control
	Sample dilution buffer
	Conjugate
	Substrate
	Wash buffer (20-fold conc.)
	Stop reagent

## 16. References

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