

## RIDASCREEN® VDZ Monitoring

**REF** G09045



## 1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® VDZ Monitoring is an enzyme linked immunosorbent assay intended for the quantitative determination of vedolizumab (VDZ, Entyvio®, anti- integrin  $\alpha_4\beta_7$ ) in human serum and plasma.

## 2. Summary and explanation of the test

### Therapeutic drug monitoring

Vedolizumab (VDZ) is a humanized monoclonal antibody that binds exclusively to the lymphocyte integrin  $\alpha_4\beta_7$ . VDZ inhibits the interaction of  $\alpha_4\beta_7$ -expressing cells with mucosal addressin cell adhesion molecule-1 on endothelial cells, thereby hampering the infiltration of the  $\alpha_4\beta_7$ -expressing cells into the gastrointestinal mucosa and gut-associated lymphoid tissue. VDZ suppresses gut inflammation and has therefore been approved for the treatment of patients with moderate to severely ulcerative colitis (UC) [1] and Crohn's disease (CD). [2,3] It has been shown that VDZ can induce clinical remission and improve the patient's quality of life.

A drug can only exert its pharmacologic effect when adequate concentrations are achieved in the circulation. The serum concentration of biologicals just before their next infusion, defined as trough concentration, has been used for therapeutic drug monitoring (TDM). Recent data on TDM have shown a positive relationship between VDZ trough serum concentrations and clinical outcomes in patients with UC and CD. [4,5,6] TDM may therefore be very instrumental to optimize treatment. RIDASCREEN® VDZ Monitoring uses highly specific monoclonal antibodies (MA-VDZ6F3 and MA-VDZ6E6), which were isolated and characterized at the KU Leuven. They detect only vedolizumab (Entyvio®). Anti-TNF drugs such as infliximab, adalimumab or golimumab are not detected in the assay.

As an example of TDM, the use of VDZ trough concentration measurements in UC and CD is described.

### Ulcerative colitis

VDZ is given at week 0, week 2 and week 6 (induction) and upon good clinical response at week 14, treatment is continued by infusions every 8 weeks (maintenance). The exposure-efficacy relationships of VDZ evaluated in GEMINI 1 revealed a positive exposure-response relationship for clinical remission, clinical response, and mucosal healing for VDZ induction therapy in UC [1]. VDZ trough concentration measurements during or shortly after induction, can be used to identify undertreated patients. It has been demonstrated that higher VDZ concentrations are associated with deep remission in patients with UC on maintenance therapy [5]. Thus, regularly checking VDZ trough concentrations during maintenance therapy may be useful to evaluate the VDZ treatment schedule.

## **Crohn's Disease**

VDZ is given at week 0, week 2 and week 6 (induction) and upon good clinical response at week 14, treatment is continued by infusions every 8 weeks (maintenance). The exposure-efficacy relationships of VDZ evaluated in GEMINI 2 and 3 revealed a modest positive exposure-response relationship. <sup>[2,3]</sup> Clinical remission rates were higher at week 10 than at week 6 in both studies. The European Medicines Agency allows an additional dose at week 10 before assessment of an induction response at week 14. Due to the dosing regimen, trough concentrations during induction at week 2, week 6, week 10 & 14 are higher compared to trough concentrations during maintenance when VDZ is given every 8 weeks.

## **Immunogenicity**

The immunogenicity rate during treatment with VDZ is very low ( $\leq 4\%$ ) and is viewed only as a minor hindrance of VDZ treatment efficacy. <sup>[5,7]</sup>

## **3. Test principle**

In RIDASCREEN® VDZ Monitoring, specific antibodies against VDZ (isolated and characterized at KU Leuven) are used in a sandwich-type method. Anti-VDZ monoclonal antibody MA-VDZ6F3 molecules are applied to the surface of the well in the microwell plate. A dilution of the patients' serum or plasma sample is pipetted into the well of the microwell plate and incubated. During this incubation step, VDZ binds specifically to MA-VDZ6F3 on the plate. After washing, a second incubation step follows with anti-VDZ monoclonal antibody MA-VDZ6E6, which is conjugated with horseradish peroxidase. In the presence of VDZ, a sandwich complex is formed between immobilized anti-VDZ antibody, VDZ and conjugated antibodies. Unattached enzyme-labelled antibodies are removed during a subsequent washing step. After addition of the substrate, the colourless solution in the microwells will turn blue in case of a positive test result. Upon addition of the stop reagent, the colour changes from blue to yellow. The absorbance is proportional to the concentration of VDZ present in the sample.

#### 4. Reagents provided

One kit is sufficient for 96 determinations.

Plate	96 det.	Microwell plate, 12 microwell strips (can be divided) in the strip holder; coated with monoclonal antibody MA-VDZ6F3
Standard  1-6	1300 µl	6 Standards; concentrations of the standards 1 to 6: 0 / 10 / 30 / 100 / 200 / 500 ng/ml; contains 0.09 % NaN <sub>3</sub> ; ready for use
Low Control  +	1300 µl	Low positive control; contains 120 ng/ml VDZ and 0.09 % NaN <sub>3</sub> ; ready for use
Control  +	1300 µl	Positive control; contains 250 ng/ml VDZ 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-VDZ6E6); 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 held for 6 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 and stored at 2 - 8°C. 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)
- 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 only.

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. The control sera of the kit (Standard 1 - 6, Low positive control, Positive control) were tested for HIV- and HCV-Ab and HBs-Ag and found to be negative. Nevertheless, they should, be treated as potentially infectious and handled according to national safety regulations, just like the patient samples and all materials coming into contact with them.

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. Avoid contact with the skin and clothing. If the skin is contaminated with the reagent, rinse it off with water.

The reagents contain NaN<sub>3</sub> as a preservative. This substance must not be allowed to come into contact with the skin or mucous membrane.

The substrate contains hydrogen peroxide.

## 8. Collection and storage of specimens

In this assay, both 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. Serum and plasma samples can be stored at 2 - 8°C for 3 - 4 days or at - 20°C for at least one year. 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 microwell 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. After use, the 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 it 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 and plasma samples can be stored at 2 - 8°C for 3 - 4 days or at - 20°C for at least one year (also see passage 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 concentration during maintenance therapy phase

To measure the trough concentration (samples taken just before next infusion) during the maintenance phase of the treatment (from week 14 and following), 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.

By diluting the samples 1:100, VDZ concentrations between 1 and 50 µg/ml can be determined.

### **b) Measurement of trough concentration during induction therapy phase**

To measure trough concentration during induction therapy (in week 2, 6 and week 14 and in Crohn's disease additionally week 10 (see chapter 2.)) or to measure intermediate concentrations, the samples are diluted 1:400:

10 µl of the sample is diluted in 390 µl sample dilution buffer [Diluent] (1:40).

Subsequently 100 µl of this solution is diluted in 900 µl [Diluent] (1:10).

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

By diluting the samples 1:400, VDZ concentrations between 4 and 200 µg/ml can be determined.

### **9.4 First incubation**

After placing a sufficient number of wells in the strip holder, add 100 µl of standards 1 to 6 [Standard | 1] to [Standard | 6], the positive control [Control | +], the low positive control [Low Control | +], and the final diluted samples. Although it is recommended to pipette the standards, controls, and samples in duplicate, reliable results are equally obtained by doing the analysis in singlicate. Then cover the plate and incubate for 60 minutes at 37°C.

### **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 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 reagent 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 the reagent stability and correct procedural.

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

OD Value for Standard 1 **Standard | 1** < 0.080

OD 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 | +**:

12 µg/ml, range 8 – 16 µg/ml

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

25 µg/ml, range 17 – 34 µg/ml

### b) If the dilution factor of 1:400 is used (induction therapy phase):

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

48 µg/ml, range 32 – 64 µg/ml

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

100 µg/ml, range 68 – 136 µg/ml

For calculating the VDZ 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 ng/ml x 100 (dilution factor) = 6 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.

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

Evaluation of RIDASCREEN® VDZ 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 VDZ 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 120 ng/ml. The corresponding VDZ concentration in the undiluted sample is then 12 µg/ml

Example: the outcome of 1:400 diluted sample, obtained by interpolation from the calibration curve is 150 ng/ml. The corresponding VDZ concentration in the undiluted sample is then 60 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 VDZ100.met.

For dilution 1:400 select: RIDASOFT® Win.NET method VDZ400.met.

The concentration is reported in µg/ml.

## 12. Limitations of the method

The RIDASCREEN® VDZ Monitoring test detects the free proportion of VDZ and not the proportion of VDZ that is bound by anti-vedolizumab antibodies, because of immunogenicity.

The RIDASCREEN® VDZ Monitoring is intended to be used as a diagnostic tool to support clinical decisions and not to replace the clinical decisions. The results of the RIDASCREEN® VDZ Monitoring should always be interpreted in conjunction with all other available patient, drug, and disease-specific parameters.

## 13. Performance characteristics

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

Standard	O.D.
1	0.011
2	0.091
3	0.232
4	0.740
5	1.323
6	2.499

### 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 VDZ concentrations have been determined. The mean value (MV), standard deviation (SD) and coefficient of variation (CV) were calculated for each sample. The results are listed in the table below.

Reference	1	2	3	4
Mean (µg/ml)	2.64	7.11	13.87	32.35
SD	0.12	0.39	0.75	2.33
<b>% CV</b>	<b>4.6</b>	<b>5.5</b>	<b>5.4</b>	<b>7.2</b>

### 13.2.2 Inter-Assay Precision

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

Reference	1	2	3	4
Mean (µg/ml)	2.67	7.29	14.31	33.42
SD	0.15	0.49	0.93	4.05
% CV	<b>5.8</b>	<b>6.7</b>	<b>6.5</b>	<b>12.1</b>

### 13.3. Specificity

#### 13.3.1. Normal human serum/plasma

Specificity has been evaluated by testing 72 healthy donor samples from Dutch and Belgian origin. None of the samples showed a detectable concentration of VDZ, resulting in a specificity of 100 %.

#### 13.3.2. Interference

Interference has been evaluated by testing 27 RF positive samples from patients suffering from autoimmune diseases, treated with medication other than Entyvio®. None of the samples showed a detectable concentration of VDZ, resulting in a specificity of 100%.

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

#### 13.3.3. Cross-reactivity

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

### 13.4. Analytical sensitivity

The limit of detection of VDZ is lower than 2.5 ng/ml. Taking into account a dilution factor of 1:100 this corresponds to 0.25 µg/ml.

### 13.5. Recovery

15 VDZ-negative samples (5 negative serum samples, 5 negative EDTA plasma samples, 5 negative citrate plasma samples) were spiked with three different VDZ-concentrations (6 µg/ml, 12 µg/ml and 40 µg/ml).

Based on the OD values of this measurement, the VDZ concentration was determined using the standard curve and the recovery calculated. The mean recovery is 98.1%.

Sample	Nr.	VDZ (µg/ml)	Recovery (%)
<b>Reference value 43.0 µg/ml</b>			
Serum	1	41.7	97.0%
	2	42.9	99.8%
	3	41.4	96.3%
	4	39.3	91.4%
	5	38.9	90.5%
EDTA Plasma	6	41.5	96.5%
	7	43.4	100.9%
	8	44.5	103.5%
	9	42.5	98.8%
	10	38.3	89.1%
Citrate Plasma	11	42.9	99.8%
	12	41.2	95.8%
	13	39.7	92.8%
	14	40.5	94.2%
	15	39.3	91.4%
<b>Reference value 10.2 µg/ml</b>			
Serum	1	10.5	102.9%
	2	9.4	92.2%
	3	10.5	102.9%
	4	10.2	100.0%
	5	10.6	103.9%

Sample	Nr.	VDZ (µg/ml)	Recovery (%)
EDTA Plasma	6	10.1	99.0%
	7	9.8	96.1%
	8	11.1	108.8%
	9	10.0	98.0%
	10	9.7	95.1%
Citrate Plasma	11	11.1	108.8%
	12	9.9	97.1%
	13	9.8	96.1%
	14	10.4	102.0%
	15	9.8	96.1%
<b>Reference value 6.5 µg/ml</b>			
Serum	1	6.0	92.3%
	2	5.7	87.7%
	3	5.7	87.7%
	4	6.4	98.5%
	5	6.9	106.2%
EDTA Plasma	6	6.2	95.4%
	7	7.6	116.9%
	8	6.3	96.6%
	9	6.7	103.1%
	10	6.2	95.4%
Citrate Plasma	11	6.4	98.5%
	12	6.2	95.4%
	13	7.4	113.8%
	14	5.7	87.7%
	15	6.8	104.6%
<b>Mean</b>			<b>98.1%</b>

### 13.6. Correlation with reference assay and diagnostic sensitivity

A clinical sample panel of 17 specimens was analyzed using the RIDASCREEN® VDZ Monitoring and results were compared with data obtained using a reference assay (KU Leuven). Pearson r value as indicator for the correlation between both assays is 0.98.










All samples having measurable VDZ levels according to the reference assay were detected positive (16 samples) resulting in a diagnostic sensitivity of 100%.

## 14. Version history

Version number	Chapter and description
2019-12-10	9.4. First incubation

## 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

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

## 16. References

1. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* 2013;369:699-710.
2. Sandborn WJ, Feagan BG, Rutgeerts P, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med.* 2013;369:711-721.
3. Sands BE, Feagan BG, Rutgeerts P, et al. Effects of vedolizumab induction therapy for patients with Crohn's disease in whom tumor necrosis factor antagonist treatment failed. *Gastroenterology* 2014;147:618-627.
4. Rosario M, Dirks NL, Milch C, et al. A Review of the Clinical Pharmacokinetics, Pharmacodynamics, and Immunogenicity of Vedolizumab. *Clin Pharmacokinet* 2017;56:1287-1301.
5. Rosario M, French JL, Dirks NL, et al. Exposure–efficacy Relationships for Vedolizumab Induction Therapy in Patients with Ulcerative Colitis or Crohn's Disease. *J Crohns Colitis* 2017;11:921-929.
6. Dreesen E, Verstockt B, Bian S, et al. Evidence to support monitoring of vedolizumab trough concentrations in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2018; *Epub ahead of print.*
7. Bian S, Dreesen E, Tang HT, et al. Antibodies toward vedolizumab appear from the first infusion onward and disappear over time. *Inflamm Bowel Dis* 2017;23:2202-2208.