

ab280327 – Daratumumab (Human) ELISA Kit

For *in vitro* quantitative determination of free Daratumumab in human serum and plasma samples.

Daratumumab (Human) ELISA kit (ab280327) measures the free Daratumumab concentration in human serum or plasma with high sensitivity and reproducibility.

Standards and samples (serum or plasma) are added to the Micro ELISA Plate coated with the reactant for Daratumumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Daratumumab captured by the reactant on the surface of the wells. Following incubation, wells are washed, and the enzymatic activity is detected by the addition of TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of Daratumumab in the sample or standard. The sample results can be determined directly using the standard curve.

Except for Daratumumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.

Recovery rate: < 100 ± 30% with known concentrations of normal human serum samples.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab280327>

Storage and Stability

The entire ELISA kit may be stored at 4°C.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	2 x 50 ml	4°C
Micro ELISA Plate	1 unit	4°C
Daratumumab Standards (S1 – S7)	7 vials	4°C
HRP-conjugate Probe	1 x 12 ml	4°C
Plate sealers	2 units	4°C
Stop Solution	1 x 12 ml	4°C
TMB substrate	1 x 12 ml	4°C
Wash buffer (20X)	1 x 50 ml	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes and tips.
- Eppendorf tubes
- Absorbent paper

Sample Preparation

Samples and reagents must be prepared freshly before the start of the experiment. Allow all reagents and samples to reach room temperature (RT). Gently swirl each sample and reagent, without foaming, prior to use.

Reagent Preparation

Wash Buffer:

Dilute 20X Wash Buffer to 1X solution in ddH₂O (10 ml of 20X Wash Buffer + 190 ml ddH₂O). To dissolve the crystals, warm the Wash Buffer at 37°C. Mix vigorously. The working stock is stable for 2 weeks after preparation at 4°C.

Standards:

Standards are supplied ready to use.

Name	S1	S2	S3	S4	S5	S6	S7
ng/ml	1000	300	100	30	0	High control	Low control

Sample Dilution

Dilute Serum/Plasma samples 1:2000 in Assay Buffer. First, make 1:20 dilution (10µl sample + 190 µl Assay Buffer). Next, prepare 1:100 dilution (5 µl previously diluted sample + 495 µl Assay Buffer).

Assay Procedure

Bring all reagents, samples and Micro ELISA Plate to room temperature (RT)

It is recommended that all standards and samples be run at least in duplicates

A standard curve must be run with each assay

1. Prepare samples (serum/plasma) and reagents as instructed.
2. Pipette 100 µl "Assay Buffer" into each of the wells to be used.
3. Add 10 µl of standards, controls and samples into the respective wells of the microtiter plate. Cover the plate with Plate sealer, gently mix the contents in the plate, and incubate at RT for 60 mins.
4. Remove the sealer and discard the incubation solution. Wash the plate 3 times with 300 µl of 1X Wash Buffer. Remove excess solution by tapping the inverted plate on an absorbent paper.
5. Add 100 µl of HRP-conjugate Probe into each well. Cover the plate and incubate at RT for 30 mins.
6. Discard the incubation solution and wash wells as mentioned in Step 4.
7. Add 100 µl of TMB Substrate into each well. Incubate the plate without plate sealer in the dark at RT for 10 mins.
8. Add 100 µl of Stop Solution to stop the reaction. Gently mix the plate. The color changes from blue to yellow.
9. Measure the absorbance using microplate reader at 450 nm within 30 minutes of adding Stop Solution. (Use reference wavelength as 650 nm).
10. Prepare a standard curve using the standards (disregard standard zero). Plot OD (450/650 nm) values for each standard on the vertical (Y-axis) axis versus the corresponding Daratumumab concentration on the horizontal (X-axis) axis. Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (1000x).

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

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