ab282906 – Aflibercept ELISA Kit

For *in vitro* quantitative determination of Aflibercept in human serum and plasma samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab282906

Materials Supplied and Storage Conditions

Item	Quantity	Storage Condition
Assay Buffer	1 x 50 ml	4°C
Aflibercept Standards S1 to S7	7 x 300 µl	4°C
HRP-conjugate Probe	1 x 12 ml	4°C
Plate sealers	2 units	4°C
Reactant for Aflibercept coated microtitre plate	1 unit	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:

- Micropipettes and tips
- Eppendorf tubes
- Absorbent paper
- Microtiter plate reader capable of measuring absorbance at 450 nm

Standard and Controls Preparation

Standards are supplied ready to use:

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

Reagent 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.

Wash Buffer:
Dilute 20X Wash Buffer to 1X solution in ddH_2O (10 ml of 20X Wash Buffer + 190 ml ddH_2O). 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.

- All other reagents are supplied ready to use.

Sample Preparation

Sample Dilution:
Dilute samples 1:100 in Assay Buffer (5 µl sample + 495 µl Assay Buffer). Diluted samples should further be diluted if the concentration of Aflibercept is higher than the measuring range. The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 2 days and 20°C for 6 months. Avoid freeze-and-thaw cycles.

Assay Procedure

- Bring all reagents, samples, and microtiter 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 standards, controls, and samples.
2. Add 100 µl Assay Buffer into all of the wells to be used, no exceptions.
3. Add 20 µl of standards, controls, and samples into appropriate wells. Cover the plate with plate sealer, gently mix the contents in the plate, and incubate at room temperature 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 room temperature 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 room temperature for 15 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).

Calculation

1. Prepare a standard curve using the standards (disregard standard zero).
2. Plot optical density (OD) (450/650 nm) values for each standard on the vertical (Y-axis) axis versus the corresponding Aflibercept concentration on the horizontal (X-axis) axis.
3. Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to point calculation curve fit.
4. To obtain the exact values of the samples, the concentration determined from the standard-curve **must be multiplied by the dilution factor.**

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

For all technical or commercial enquiries please go to:
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