ab237653 – Ipilimumab ELISA Kit

For the quantitative analysis of free ipilimumab 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/ab237653

Storage and Stability

On receipt, the entire kit should be stored at 4°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	2 x 50 mL	4°C
HRP-Conjugate	12 mL	4°C
Ipilimumab Standard S1	0.3 mL	4°C
Ipilimumab Standard S2	0.3 mL	4°C
Ipilimumab Standard S3	0.3 mL	4°C
Ipilimumab Standard S4	0.3 mL	4°C
Ipilimumab Standard S5	0.3 mL	4°C
Ipilimumab Standard S6	0.3 mL	4°C
Ipilimumab Standard S7	0.3 mL	4°C
Micro ELISA Plate	12 x 8 strips	4°C
Plate sealers	2 units	4°C
Stop Solution	12 mL	4°C
TMB substrate	12 mL	4°C
Wash buffer (20X)	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
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Immunogen coated ELISA Plate: Do not open until ready to use. Bring to room temperature (RT) prior to opening. After opening, immediately store the remaining unused antibody-coated strips at 4°C in the foil bag.

Ipilimumab Standards (S1 – S7): Ready to use. Store at 4°C.

Assay Buffer, TMB Substrate and Stop Solution: Provided as a ready-to-use solution. Bring to RT prior to use. When not in use, reseal the bottle immediately and store at 4°C, protected from light.

HRP Conjugate: Divide the stock into aliquots, if desired and store at 4°C, protected from light. Avoid repeated freeze-thaw cycles.

Wash Buffer (20X): Warm to RT prior to use (if crystals are present, mix gently until the crystals are completely dissolved). Prepare 1X Wash Buffer solution by diluting Wash Buffer (20X) with deionized water (i.e. mix 10 ml of 20X Wash Buffer with 190 ml of ddH_2O and mix well). The 1X Wash Buffer is stable at 4°C for 2 weeks.

Standard Preparation

- Ready to use (S1-S7)

Standard	S1	S2	S3	S4	S5	S6	S7
Concentration (ng/ml)	300	100	30	10	0	High Control	Low Control

Sample Preparation

Serum/Plasma: Prepare 1:300 dilution of the samples in Assay Buffer. First, prepare 1:10 dilution (10 µl of sample + 90 µl Assay Buffer). Then prepare 1:30 dilution (30 µl of previously diluted sample + **870** µl Assay Buffer).

- Diluted samples should further be diluted if the concentration of Ipilimumab is higher than the Standard Curve range.
- Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Assay Protocol

- Bring all the reagents, microplate and samples to room temperature (RT), 15 min prior to the assay. All Standards and samples should be run at least in duplicates. A Standard Curve must be run with each assay. Prepare all the reagents, samples and Standards as instructed.

1. Pipette 100 µl of Assay Buffer into each of the well to be used.
2. Add 10 µl of each Standards, Controls and diluted samples into the appropriate wells.
3. Cover the plate with a plate sealer and incubate for 30 min at RT.
4. Remove the plate sealer. Aspirate the solution from each well and wash each well 3 times with 300 µl of 1X Wash Buffer. Remove the 1X Wash buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
5. Add 100 µl of HRP-conjugate into each well and mix well. Cover the plate with a plate sealer and incubate at RT for 30 min.
6. Remove the plate sealer. Aspirate all reagents and wash each well 3 times with 300 µl of 1X Wash Buffer. Remove the 1X Wash buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
7. Add 100 µl of TMB substrate solution to all wells and incubate at RT for 10 min in the dark.
8. Add 100 µl of Stop solution to all wells and measure the absorbance at 450 nm within 30 min in micro plate reader (use reference wavelength as 650 nm).

Calculation

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 Ipilimumab 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 should be multiplied by the dilution factor (300x).

Technical Support

For all technical or commercial enquiries please go to:
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