ab285345 – Human Progranulin ELISA Kit

For in vitro quantitative determination of Human Progranulin in human serum, plasma, and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285345

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 6 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Plate coated with human Progranulin antibody	6 x 16-wells strips	4°C
Wash Buffer (10x)	30 mL x 2	4°C
ELISA Buffer (10X)	30 mL x 2	4°C
Detection Antibody (DET)	30 µL	4°C
HRP Labeled Streptavidin (lyophilized)	2 µg	4°C
Human progranulin Standard (lyophilized)	8 ng	4°C
TMB Substrate Solution	12 mL	4°C
Stop Solution	12 mL	4°C
Plate sealers (plastic film)	2 units	4°C
Silica Gel Minibags	2 units	4°C

Materials Required, Not Supplied

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

- Microtiterplate reader at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Calibrated precision single and multi-channel pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

Reagent Preparation

- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

Wash Buffer 10X: Dilute with deionized water 1:10 before use (e.g., 50 ml Wash Buffer 10X + 450 ml water) to obtain Wash Buffer 1X.

ELISA Buffer 10X: Dilute with deionized water 1:10 before use (e.g., 20 ml ELISA Buffer 10X + 180 ml water) to obtain ELISA Buffer 1X.

Detection Antibody (DET): Dilute to 1:1000 in ELISA Buffer 1X (10 µl DET + 10 ml ELISA Buffer 1X).

Δ Note: *The diluted Detection Antibody is not stable and cannot be stored.*

HRP Labeled Streptavidin (STREP-HRP): Reconstitute with 100 µl of ELISA Buffer 1X. After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. Avoid freeze/thaw cycles. Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200). The diluted STREP-HRP is not stable and cannot be stored

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Standard Preparation

1. Reconstitute the standard by adding 1 mL of deionized water to make the 8 ng/mL standard stock solution. Use within 2 hours after reconstituting.
2. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions. **NOTE**: The reconstituted standard is aliquoted and stored at -20°C
3. Dilute the standard protein concentrate (STD) (8 ng/ml) in Diluent 1X. A seven-point standard curve using 2-fold serial dilutions in Diluent 1X is recommended.
4. Suggested standard points are: 4, 2, 1, 0.5, 0.25, 0.125, 0.063 and 0 ng/ml.

Sample Preparation

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.

Urine: Aseptically collect the urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at ≤ - 20°C. Avoid repeated freeze/thaw cycles.

Δ Note: *Serum, Plasma, Urine, Cell Culture Supernatant must be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use. As a starting point, 1/200 dilution of serum or plasma and 1/20 dilution of urine are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required! For CSF a starting dilution of 1/4 - 1/5 is recommended based on literature references using this ELISA Kit.*

Assay Procedure

1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips should be resealed in the foil pouch bag and stored at 4°C. **NOTE**: Remaining 16-well strips coated with progranulin antibody when opened can be stored at 4°C for up to 1 month.

2. Add 100 µl of the different standards into the appropriate wells in duplicate. At the same time, add 100 µl of diluted serum, plasma, urine, cell culture supernatant samples in duplicate to the wells. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
3. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
4. Add 100 µl to each well of the Detection Antibody. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
5. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
6. Add 100 µl to each well of the diluted HRP Labeled Streptavidin. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.
8. Add 100 µl to each well of TMB Substrate Solution. Allow the color reaction to develop at room temperature in the dark for 10 minutes.
9. Stop the reaction by adding 100 µl of Stop Solution. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
10. Measure the OD at 450 nm in an ELISA reader within 30 minutes.

Calculations

Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point). Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding progranulin concentration (ng/ml) on the vertical (Y) axis (see Typical Data). Calculate the progranulin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation. If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human progranulin in the samples.

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

For technical support contact information, visit: www.abcam.com/contactus