# ab284622 – Human FGL1 SimpleStep ELISA® Kit

For the quantitative measurement of FGL1 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, cell extract, and tissue extract.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:** www.abcam.com/ab284622

**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Human FGL1 Capture Antibody 10X | 600 µL | +4°C |
| Human FGL1 Detector Antibody 10X | 600 µL | +4°C |
| Human FGL1 Lyophilized Recombinant Protein | 2 Vials | +4°C |
| Antibody Diluent 5Bl | 6 mL | +4°C |
| Cell Extraction Buffer PTR 5X | 10 mL | +4°C |
| Cell Extraction Enhancer Solution 50X | 1 mL | +4°C |
| Sample Diluent NS | 50 mL | +4°C |
| Wash Buffer PT 10X | 20 mL | +4°C |
| TMB Development Solution | 12 mL | +4°C |
| Stop Solution | 12 mL | +4°C |
| SimpleStep Pre-Coated 96-Well Microplate | 96 wells | +4°C |
| Plate Seal | 1 | +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 or 600 nm.
   Method for determining protein concentration (BCA assay recommended).
   Multi- and single-channel pipettes.
   Tubes for standard dilution.
   Plate shaker for all incubation steps.
   Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

## Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

**1X Cell Extraction Buffer PTR (For cell and tissue extracts only):** Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X.  Mix thoroughly and gently. If required protease inhibitors can be added.

**1X Wash Buffer PT:** Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

**Antibody Cocktail:** Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5Bl. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5Bl. Mix thoroughly and gently.

## Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

**IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the FGL1 standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the FGL1 standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently.  This is the 10,000 pg/mL **Stock Standard** Solution.

1. For **serum**, **plasma, and cell culture supernatant samples measurements**, reconstitute the FGL1 standard by adding Sample Diluent NS. For **cell and tissue extract samples measurements**, reconstitute the FGL1 standard by adding 1X Cell Extraction Buffer PTR.
2. Label eight tubes, Standards 1– 8.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 280 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

| Standard # | Dilution Sample | Volume to Dilute (µL) | Volume of Diluent (µL) | Starting Conc. (pg/mL) | Final Conc. (pg/mL) |
|---|---|---|---|---|---|
| 1 | Stock Standard | 120 | 280 | 10,000 | 3,000 |
| 2 | Standard#1 | 150 | 150 | 3,000 | 1,500 |
| 3 | Standard#2 | 150 | 150 | 1,500 | 750 |
| 4 | Standard#3 | 150 | 150 | 750 | 375 |
| 5 | Standard#4 | 150 | 150 | 375 | 187.5 |
| 6 | Standard#5 | 150 | 150 | 187.5 | 93.75 |
| 7 | Standard#6 | 150 | 150 | 93.75 | 46.88 |
| 8 | Blank Control | 0 | 150 | N/A | N/A |

| Typical Sample Dynamic Range | |
| --- | --- |
| Sample Type | Range |
| Serum | 0.063 - 1% |
| Plasma - Citrate | 0.016 - 0.5% |
| Plasma - EDTA | 0.016 - 0.5% |
| Plasma - Heparin | 0.016 - 0.5% |
| HepG2 cell culture supernatant | 0.031 - 0.5% |
| HepG2 Cell extract | 7.81 - 125 µg/mL |
| A549 Cell extract | 62.5 - 500 µg/mL |
| Liver Tissue extract | 125 - 500 µg/mL |

**Serum** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:100 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

**Plasma** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatants** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

**Preparation of extracts from cell pellets** Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC. Rinse cells twice with PBS. Solubilize pellet at 2x10⁷ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

**Preparation of extracts from tissue homogenates** Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4ºC. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80ºC. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4ºC.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

## Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate
1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

| Mode | Kinetic |
| --- | --- |
| Wavelength: | 600 nm |
| Time: | up to 20 min |
| Interval: | 20 sec - 1 min |
| Shaking: | Shake between readings |

**Note** that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

**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

# ab284622 – Human FGL1 SimpleStep ELISA® Kit

## Additional information

### ASSAY SPECIFICITY

This kit is designed for the quantification of human FGL1.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, cell extract, and tissue extract.

Saliva, urine, milk, and cerebrospinal fluid samples have not been tested with this kit.

### SPECIES REACTIVITY

Other species reactivity was determined by measuring 0.25% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

This kit is 57% reactive in mouse serum samples and 15% reactive in rat serum samples

Reactivity < 3% was determined for the following species: Monkey, Cow

Other species reactivity not determined.

### CALCULATION
- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

## TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.056 | 0.056 | 0.056 |
| 46.88 | 0.135 | 0.133 | 0.134 |
| 93.75 | 0.217 | 0.211 | 0.214 |
| 187.5 | 0.383 | 0.366 | 0.374 |
| 375 | 0.569 | 0.567 | 0.568 |
| 750 | 1.028 | 1.024 | 1.026 |
| 1,500 | 2.031 | 2.028 | 2.030 |
| 3,000 | 3.259 | 3.256 | 3.257 |

Table 1. Example of human FGL1 standard curve in Sample Diluent NS. The FGL1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.071 | 0.071 | 0.071 |
| 46.88 | 0.150 | 0.128 | 0.139 |
| 93.75 | 0.208 | 0.184 | 0.196 |
| 187.5 | 0.307 | 0.273 | 0.290 |
| 375 | 0.490 | 0.469 | 0.479 |
| 750 | 0.842 | 0.839 | 0.840 |
| 1,500 | 1.553 | 1.602 | 1.578 |
| 3,000 | 2.965 | 3.150 | 3.058 |

Table 2. Example of human FGL1 standard curve in 1X Cell extraction Buffer PTR. The FGL1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

## TYPICAL SAMPLE VALUES

### Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

| Sample Diluent Buffer | N= | Minimal Detectable Dose |
|---|---|---|
| Sample Diluent NS | 24 | 21.73 pg/mL |
| 1X Cell Extraction Buffer PTR | 24 | 20.08 pg/mL |

### Recovery

Three concentrations of FGL1 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| 0.25% Serum | 106 | 95 - 117 |
| 0.25% Plasma - Citrate | 96 | 87 - 103 |
| 0.25% Plasma - EDTA | 108 | 102 - 113 |
| 0.25% Plasma - Heparin | 95 | 92 - 99 |
| 0.25% HepG2 Cell Culture Supernatant* | 105 | 96 - 118 |
| 31.25 µg/mL HepG2 Cell Extract | 96 | 85 - 104 |
| 62.5 µg/mL A549 Cell Extract | 106 | 90 - 122 |
| 125 µg/mL Liver Tissue Extract | 113 | 106 - 118 |

*Media is DMEM containing 10% fetal calf serum

### Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native FGL1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

| Dilution Factor | Interpolated value | 1% Human Serum | 0.5% Human Plasma (Citrate) | 0.5% Human Plasma (EDTA) | 0.5% Human Plasma (Heparin) | 0.5% HepG2 Cell Supernatant |
|---|---|---|---|---|---|---|
| Undiluted | pg/mL | 786.35 | 1952.91 | 2336.89 | 2123.77 | 1681.04 |
| | % Expected value | 100 | 100 | 100 | 100 | 100 |
| 2 | pg/mL | 409.97 | 1008.67 | 1147.30 | 1069.21 | 775.00 |
| | % Expected value | 104 | 103 | 98 | 101 | 92 |
| 4 | pg/mL | 218.47 | 499.62 | 582.99 | 527.71 | 413.76 |
| | % Expected value | 111 | 102 | 100 | 99 | 98 |
| 8 | pg/mL | 111.37 | 261.70 | 305.45 | 264.77 | 206.48 |
| | % Expected value | 113 | 107 | 105 | 100 | 98 |
| 16 | pg/mL | NL | 130.99 | 145.74 | 139.22 | 105.07 |
| | % Expected value | NL | 107 | 100 | 105 | 100 |

NL – not linear

Native FGL1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

| Dilution Factor | Interpolated value | 250 µg/mL HepG2 Cell Extract | 500 µg/mL A549 Cell Extract | 500 µg/mL Human Liver Tissue Extract |
|---|---|---|---|---|
| Undiluted | pg/mL | 2132.38 | 487.23 | 240.45 |
| | % Expected value | 100 | 100 | 100 |
| 2 | pg/mL | 1142.01 | 266.84 | 114.17 |
| | % Expected value | 107 | 110 | 95 |
| 4 | pg/mL | 565.49 | 138.77 | 51.34 |
| | % Expected value | 106 | 114 | 85 |
| 8 | pg/mL | 284.62 | 65.76 | ND |
| | % Expected value | 107 | 108 | ND |
| 16 | pg/mL | 151.90 | ND | ND |
| | % Expected value | 114 | ND | ND |

ND – Not Detected – below product dynamic range

### Precision

Mean coefficient of variations of interpolated values of FGL1 from three concentrations of serum within the working range of the assay.

| | Intra-assay | Inter-assay |
|---|---|---|
| N= | 8 | 3 |
| CV (%) | 4.3 | 4.5 |

**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