# Mouse Slit Homolog 2 (Slit2) ELISA Kit

**Catalog No.:** abx051309

**Size:** 96T

**Range:** 16 - 1000 pg/ml

**Sensitivity:** 6.5 pg/ml

**Storage:** Store at 2-8° C for 6 months.

**Application:** For quantitative detection of Slit2 in Mouse serum, plasma, cell culture supernatant or any biological fluid.

**Introduction**

The main function of Slit proteins is to act as midline repellents, preventing the crossing of longitudinal axons through the midline of the central nervous system of most bilaterian animal species, including mice, chickens, humans, insects, nematode worms and planarians. It also prevents the recrossing of commissural axons. Its canonical receptor is Robo but it may have other receptors. The Slit protein is produced and secreted by cells within the floor plate (in vertebrates) or by midline glia (in insects) and diffuses outward.

**Principle of the Assay**

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-Slit2 antibody is pre-coated onto 96-well plates. An HRP conjugated anti-Slit2 antibody is used as detection antibody. The standards, test samples and HRP conjugated detection antibody are added to the wells and incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the amount of Slit2 sample captured in plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of Slit2 can be calculated.

**Kit components**

1. One 96-well plate pre-coated with anti-Mouse Slit2 antibody
2. Mouse Slit2 Standard: 0.5 ml
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1/30
5. Sample diluent buffer: 6 ml
6. HRP conjugated anti-Mouse Slit2 antibody (RTU): 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6ml
9. TMB substrate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

**Material Required But Not Provided**

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions.
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders.

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## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

✧ **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Serum:** Samples should be collected into a serum separator tube. Incubate at room temperature (~2 hour) or overnight at 4°C. Centrifuge at 2000-3000 × g for 10 - 20 min. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Plasma:** Collect plasma with citrate or EDTA as the anticoagulant. Mix for 20 min approximately. Centrifuge at 2000-3000 × g for 20 min within 30 min of collection. Analyze immediately or aliquot and store at -20°C or -80°C.

✧ **Urine:** Collect in a sterile container. Centrifuge at 10,000 × g for 2 min at 4°C.

✧ **Tissues:** The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize in PBS with a tissue homogenizer on ice. Homogenate completely and centrifuge to collect the supernatant. Analyze immediately or aliquot and store at -20°C or -80°C.

**Note:**

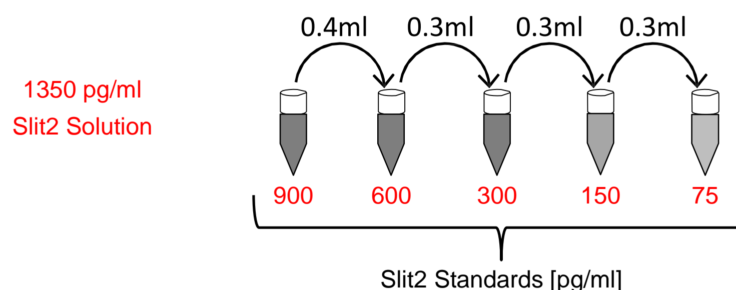
- Samples must be diluted so that the expected concentration falls within the kit's range.
- Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant. Hemolysis will influence the result. Please bring samples slowly to room temperature.
- $NaN_3$ cannot be used as test sample preservative, since it inhibits HRP.

#### 2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

#### 3. Standard

Label 5 tubes with 900 pg/ml, 600 pg/ml, 300 pg/ml, 150 pg/ml and 75 pg/ml respectively. Aliquot **0.2 ml** of the Standard diluent buffer into the first two tubes labeled 900 pg/ml and 600 pg/ml respectively and **0.3 ml** of the Standard diluent buffer into each remaining tube. Add **0.4 ml** of 1350 pg/ml standard solution into 1st tube and mix thoroughly. Transfer **0.4 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on. The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.



**B. Assay Procedure**

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, record their positions. It is recommended to assay standards, samples and controls in duplicate.
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Aliquot 50 µl of Standard diluent buffer into the control (zero) well. Do not add sample and HRP conjugated antibody into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample (Mouse serum, plasma or cell culture supernatants) into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
5. Seal the plate with a cover and incubate at 37°C for 30 min.
6. Remove the cover and wash the plate 5 times with one of the following methods.

**Manual Washing:** Discard the solution without touching the side walls. Tap the plate on absorbent paper or other absorbent material. Fill each well completely with wash buffer and vortex mildly on ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure five times.

**Automated Washing:** Discard the solution in all wells and wash the plate five times with Wash buffer (overfilling the wells with the buffer). After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

7. Aliquot 50 µl of HRP conjugated anti-Slit2 antibody into each well (except control well).
8. Seal the plate with a cover and incubate at 37°C for 30 min.
9. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.
10. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
11. Aliquot 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader within 15 min of adding the stop solution.

For calculation, (relative $O.D._{450}$) = ($O.D._{450}$ of each well) – ($O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Mouse Slit2 concentration of the samples can be interpolated from the standard curve.

**Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

**C. Precautions**

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. It is recommend to measure each standard and sample in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. To avoid cross contamination do not reuse pipette tips and tubes.
7. Do not use expired components or components from a different kit.
8. Store the TMB substrate B in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrates for 30 min at room temperature prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

**D. Precision**

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Mouse Slit2 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high Mouse Slit2 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

**E. Typical Data & Standard Curve**

Results of a typical standard run of a Mouse Slit2 ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

| pg/ml | 0 | 75 | 150 | 300 | 600 | 900 |
|---|---|---|---|---|---|---|
| OD450 | 0.027 | 0.195 | 0.368 | 0.782 | 1.396 | 1.984 |