

## Human Substance P CLIA Kit

**Catalog No.:** abx190100

**Size:** 96T

**Range:** 7.81 pg/ml - 2000 pg/ml

**Sensitivity:** < 2.86 pg/ml

**Storage:** Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4°C.

**Application:** For quantitative detection of SP in Human Serum, Cell Culture Supernatants, Plasma, Tissue Homogenates, Cell Lysates and other biological fluids.

**Introduction:** Substance P (SP) is an undecapeptide (a peptide composed of a chain of 11 amino acid residues) member of the tachykinin neuropeptide family. It is a neuropeptide, acting as a neurotransmitter and as a neuromodulator. Substance P and its closely related neurokinin A (NKA) are produced from a polyprotein precursor after differential splicing of the preprotachykinin A gene. The deduced amino acid sequence of substance P is as follows: Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met (RPKPQQFFGLM) with an amidation at the C-terminus. Substance P is released from the terminals of specific sensory nerves. It is found in the brain and spinal cord and is associated with inflammatory processes and pain.

### Principle of the Assay

This kit is based on chemiluminescent immunoassay technology. Anti-SP antibody is pre-coated onto 96-well plates. The standards and samples are added to the wells with a biotin conjugated anti-SP and incubated. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After the mixture of substrate A and B is added only wells that contain SP will produce chemiluminescence. The intensity of the emitted light is proportional to the amount of SP in the sample or standard.

### Kit components

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent A: 12 ml
8. Diluent B: 12 ml
9. Substrate A: 10 ml
10. Substrate B: 2 ml
11. Plate sealer: 4

### Material Required But Not Provided

1. 37°C incubator
2. Luminometer capable of reading 96-well microplates (lag time 30.0 secs and read time 1.0 sec/well)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **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.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type - this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

#### Note:

- » Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.
- » Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2).
- » If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.
- » Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

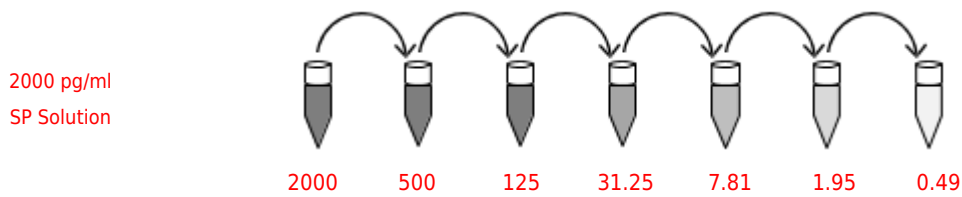
#### 2. Wash buffer

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

Bring samples and all kit components to room temperature. Prepare the Standard with 0.5ml of Standard Diluent buffer to make the 2000 pg/ml Standard Solution (use within one hour), which serves as the highest standard. Mix gently and avoid foaming or bubbles. Label 6 tubes with 500 pg/ml, 125 pg/ml, 31.25 pg/ml, 7.81 pg/ml, 1.95 pg/ml, and 0.49 pg/ml respectively. Aliquot 0.7 ml of the Standard diluent buffer into each tube. Add 0.3 ml of 2000 pg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube, mix thoroughly, and so on.

0.3 ml    0.3 ml    0.3 ml    0.3 ml    0.3 ml    0.3 ml



#### 4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with the Diluent A and B and mixed thoroughly, they are sticky solutions therefore pipette with a slow, smooth action to reduce volume errors. The solution should be prepared no more than 2 hours prior to the experiment. Reconstituted Standard, Detection Reagent A and B can only be used once.

#### 5. Substrate working solution Preparation

Substrate A and B should be mixed with a ratio of 99:1 respectively and mixed thoroughly. For example, prepare 1 ml of substrate working solution by mixing 0.99 ml of Substrate A and 0.01 ml of Substrate B.

#### B. Assay Procedure

Equilibrate the kit components and samples to room temperature before 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, and then, record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100  $\mu$ l of the diluted standards into the standard wells. Aliquot 100  $\mu$ l Standard Diluent Buffer to the control (zero) well.
3. Add 100  $\mu$ l of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Seal the plate with a cover and incubate for 1 h at 37°C.
5. Remove the cover and discard the liquid of each well. Do not wash.
6. Aliquot 100  $\mu$ l of the detection Reagent A working solution to each well. Seal the plate with a cover and incubate for 1 h at 37°C.
7. Discard the solution and wash the plate 3 times with wash buffer. Do not let the wells completely dry at any time.

**Manual Washing:** Discard the solution without touching the side walls. Tap the plate on absorbent filter papers 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 three times.

**Automated Washing:** Discard the solution and wash the plate three times overfilling the wells with Wash 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.

8. Aliquot 100  $\mu$ l of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
9. Discard the solution and wash the plate 5 times with wash buffer (each time let the Wash Buffer stay for 1-2 min). Discard the Wash Buffer and blot the plate onto paper towels or other absorbent material.
10. Aliquot 100  $\mu$ l of Substrate working solution into each well. Seal the plate with a cover and incubate at 37°C for 10 min.

11. Measure the chemiluminescence signal in a microplate luminometer immediately.

For calculation, (the relative RLU) = (the RLU of each well) - (the RLU of Zero well). The standard curve can be plotted as the relative RLU of each standard solution (Y) vs. the respective concentration of the standard solution (X). The SP concentration of the samples can be interpolated from the standard curve.

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

### C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components.
4. It is recommended measuring each standard and sample in duplicate.
5. Do NOT let the wells uncovered for extended periods between incubation. Once reagents are added to the wells DO NOT let the strips dry at any time during the assay. This can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components or components from a different kit.
10. Store the substrate A and B in the dark.

### D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of SP were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of SP were tested on 3 different plates, 8 replicates in each plate.

$$CV (\%) = SD/mean \times 100$$

Intra-Assay: CV < 10%

Inter-Assay: CV < 12%