NEUROMICS

Sample Sandwich ELISA Protocol

Components Used

- Pre-coated, stabilized 96-well microtiter plate
- Sample diluent
- Assay diluent
- Calibrator diluent
- Standards and controls
- Conjugated detection antibody (in a working dilution)
- 10X wash solution
- Single component substrate
- Stop solution

Pre-Coated, Stabilized 96-well Microtiter Plate: For microtiter plate assays, the plates are provided ready-to-use. They are pre-coated with the capture antibody, blocked, stabilized, and packaged in a resealable foil pouch with a desiccant packet. Using our methods, the plates may have a real time stability of 1 year when stored at 2^{o} – 8^{o} C. With a standard curve (1 blank and 7 standards) and 3 controls, a 96-well microtiter plate format can test 21 samples in triplicate and 37 samples in duplicate (also see Plate Coating Buffer and Blocking Buffers for more information).

Sample Diluent: The sample diluent is used to ensure that the analyte in the sample matrix is measured accurately. It is used to dilute the sample within the target range of the assay (also see Sample Diluents for more information).

Standards and controls: A known amount of the analyte (such as 1 ng) is included with a kit and run on every plate. The standard is often lyophilized. Once reconstituted, it is diluted several times to prepare a range of known values with which to compare and properly quantitate the unknown amount of analyte in a sample. Controls are used to confirm the readings of the standard and to compare readings from different plates. Because they contain a known amount of analyte, they should always read within a certain optical density (OD) value based on the standard.

Assay Diluent: In addition to a specimen diluent, certain matrixes require the use of a special assay diluent (which is applied to the plate just prior to adding the samples, standards, and controls). Assay diluents are often paired with a specific type of sample (such as serum, or cell culture media) to eliminate interference and non-specific binding generated from the matrix of the sample itself. These interferences are especially noticeable when running neat samples. An assay diluent may not be required for every assay

Calibrator Diluent: A calibrator diluent is used to ensure that the standards and controls will be measured accurately. This diluent must compliment the target analyte, capture antibody, and resemble the matrix of the sample (also see Sample Diluents for more information).

Conjugated Detection Antibody: For some assays, the detection or 'top' antibody is often an affinity purified polyclonal antibody conjugated to HRP. The enzyme-antibody conjugate can often be supplied ready-to-use in its working concentration in a special conjugate diluent. The conjugate diluent stabilizes the conjugate and minimizes nonspecific binding of the conjugate onto the blocked plate or matrix residue. The working conjugate will often have a real-time stability of 1 year when stored at 2^{o}–8^{o} C (also see Conjugate Diluents for more information).

10X Wash Solution: This specially formulated buffer is used to rinse the plate after the sample and conjugate incubation periods, just prior to the addition of the next reagent. It minimizes matrix residue and non-specific binding interferences of the samples and conjugate (also see Wash Buffer for more information).

Single Component Substrate: This reagent reacts with HRP to generate a colored signal product. It can come in many formulations (powdered, 2-component liquid, etc.). We recommend a low-background, high-signal-generating ABTS or TMB that needs no preparation prior to use.

Stop Solution: An appropriate stop solution is added to the plate with the ABTS or TMB substrate and stops its reaction with HRP. By stopping this reaction after 20 minutes, the plates can equilibrate before reading, which increases the accuracy and sensitivity of the assay.

Preparation

In this example, 1 blank (which is simply the sample diluent), 7 standards, 3 controls, and 37 unknown samples are being tested in duplicate in 1 microtiter plate MoAb-PoAb sandwich immunoassay.

Standards

As each kit typically comes with only 1 vial of a high-concentrate standard which is lyophilized (freeze dried into a powder), it must be reconstituted, and then further diluted with sample diluent. If the lyophilized standard was at 5000pg, when reconstituted with 5mL, it would have a concentration of 5000pg/5mL or 1000pg/mL. To generate a standard curve, it should be serially diluted 1:2 (500uL standard with 500uL sample diluent) to create standards at 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.25pg/mL, and 15.13pg/mL.

Controls

The controls may or may not come with a kit, but are often lyophilized and must also be reconstituted, typically with 2mL sample diluent. Controls are usually not diluted further.

Unknown Samples

Any samples thought to have a value higher than the reconstituted standard (at 1000pg/mL) should be diluted with the sample diluent so that it falls between the high standard (at 1000pg/mL) and the low standard (at 15.13 pg/mL). If they do not need to be diluted, samples often can be run neat. However, if the samples have some sort of interfering substance, such as rheumatoid factors, or complement, they may need to be treated prior to running in the assay. If you do not know about your samples, simply run them in the assay once and see where they fall (neat and diluted samples can be run at the same time). If the values are too high, just dilute, and/or treat the samples and run the assay again. Each investigator must determine their own protocols for sample dilutions and pre-assay treatments.

Immunoassay Procedure

Step 1: Add 50 uL per well of assay diluent into every well of the plate.
Step 2: Add 200 uL per well of your blanks, standards, controls, or samples onto the plate. Each item should be tested in duplicate (in 2 wells).
Step 3: Cover with plate sealer and incubate 2 hours at room temperature.
Step 4: Wash plate by filling wells with 400 uL wash buffer and dumping. Wash for a total of 4 cycles. Blot on paper towels.
Step 5: Add 200uL per well of PoAb-HRP conjugate solution into every well of the plate.
Step 6: Cover with plate sealer and incubate 2 hours at room temperature.
Step 7: Wash plate by filling wells with 400 uL wash buffer and dumping. Wash for a total of 4 cycles. Blot on paper towels.
Step 8: Add 200uL per well of TMB substrate solution into every well of the plate.
Step 9: Incubate 20 minutes at room temperature.
Step 10: Add 50 uL per well 2N HCl stop solution into every well of the plate.
Step 11: Read plate at 450nm while subtracting a reference wavelength of 540nm.
Step 12: Calculate data based on OD values of the unknown samples compared to the known values of the standard curve.

Typical Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Lo C	Lo C	Sp 6	Sp 6	Sp 14	Sp 14	Sp 22	Sp 22	Sp 30	Sp 30
B	Std 1	Std 1	Mid C	Mid C	Sp 7	Sp 7	Sp 15	Sp 15	Sp 23	Sp 23	Sp 31	Sp 31
C	Std 2	Std 2	Hi C	Hi C	Sp 8	Sp 8	Sp 16	Sp 16	Sp 24	Sp 24	Sp 32	Sp 32
D	Std 3	Std 3	Sp 1	Sp 1	Sp 9	Sp 9	Sp 17	Sp 17	Sp 25	Sp 25	Sp 33	Sp 33
E	Std 4	Std 4	Sp 2	Sp 2	Sp 10	Sp 10	Sp 18	Sp 18	Sp 26	Sp 26	Sp 34	Sp 34
F	Std 5	Std 5	Sp 3	Sp 3	Sp 11	Sp 11	Sp 19	Sp 19	Sp 27	Sp 27	Sp 35	Sp 35
G	Std 6	Std 6	Sp 4	Sp 4	Sp 12	Sp 12	Sp 20	Sp 20	Sp 28	Sp 28	Sp 36	Sp 36
H	Std 7	Std 7	Sp 5	Sp 5	Sp 13	Sp 13	Sp 21	Sp 21	Sp 29	Sp 29	Sp 37	Sp 37