Sandwich ELISA Protocol

Option 1: Using ELISA Buffer Kit

Included in ELISA Matched Antibody Pair Kit

Recombinant Protein
Antigen Affinity Purified Polyclonal or Monoclonal Antibody
Biotinylated Antigen Affinity Purified Polyclonal Antibody
Streptavidin-HRP Conjugate

Note: Standard and Antibodies should be reconstituted according to the Certificate of Analysis that accompanies each product.

Included in ELISA Buffer Kit (Cat. No. 41-9245-KIT)

PBS: 20 ml per bottle 20x concentrated PBS solution
Wash Buffer*: 500ml per bottle 20x concentrated Wash Buffer (1.0% TWEEN-20 in PBS)
Blocking Buffer: 400ml per bottle ready-to-use Blocking Buffer (1% BSA in PBS)
Diluent*: 30ml per bottle 20x concentrated Diluent (1.0% TWEEN-20, 2.0% BSA in PBS)
TMB Liquid Substrate*: 110ml per bottle ready-to-use TMB
ELISA Microplates: 10 x 96 well plates
Sealing Film: 50 polyester adhesive sheets
Stop Solution: 110ml bottle of ready-to-use Stop Solution (1M HCl)

** Light sensitive*

Preparation and Storage

Prepare buffers prior to use as indicated in the protocol. All solutions should be at ambient temperature prior to use. If crystallization occurs, gently warm and swirl the solution until crystals dissolve.

Note: The pH of the PBS, Wash Buffer, and Diluent should adjust to pH 7.2. If the pH is high, lower it using Phosphoric Acid drop wise until pH 7.2 is reached. If the pH is low, raise it by adding Sodium Hydroxide drop wise until pH 7.2 is reached.

ELISA Microplates:
Store in a clean, dust free area at ambient temperature.

Sealing Film:
Store in a clean, dry area at ambient temperature.

PBS:
Store 20x PBS at 4ºC.

Bring 20x PBS to ambient temperature and dilute to 1x PBS prior to use. Determine the required volume by following chart for desired number of plates to be tested. The pH of the working solution should adjust to pH 7.2. See note to adjust pH. 1x PBS can be stored for up to one week at 4ºC.

PBS		
Number of Plates	**20x PBS (ml)**	**Distilled Water (ml)**
1	1.5	28.5
2	3	57
3	4.5	85.5
4	6	114
5	7.5	142.5
6	9	171
7	10.5	199.5
8	12	228
9	13.5	256.5
10	15	285

Wash Buffer:

Store 20x Wash Buffer at 4ºC (light sensitive).

Bring 20x Wash Buffer to ambient temperature and dilute to 1x Wash Buffer prior to use. Determine the required volume by following chart for desired number of plates to be tested. The pH of the working solution should adjust to pH 7.2. See note to adjust the pH. 1x Wash Buffer can be stored for up to one week at 4ºC.

Wash Buffer		
Number of Plates	**20x Wash Buffer (ml)**	**Distilled Water (ml)**
1	50	950
2	100	1900
3	150	2850
4	200	3800
5	250	4750
6	300	5700
7	350	6650
8	400	7600
9	450	8550
10	500	9500

Blocking Buffer:

Store Blocking Buffer at 4ºC.

Blocking Buffer is at a working concentration. Sterile filter daily the desired volume to be used and bring to ambient temperature prior to use. To calculate the volume, multiply the number of plates by 30 ml.

Diluent:

Store 20x Diluent Buffer at 4ºC (light sensitive).

Bring 20x Diluent to ambient temperature and dilute to 1x Diluent prior to use. Determine the required volume by following chart for desired number of plates to be tested. The pH of the working solution should adjust to pH 7.2. See note to adjust pH. Sterile Filter 1x Diluent daily. 1x Diluent can be stored for up to one week at 4ºC.

Diluent		
Number of Plates	**20x Diluent (ml)**	**Distilled Water (ml)**
1	2.5	47.5
2	5	95
3	7.5	142.5
4	10	190
5	12.5	237.5
6	15	285
7	17.5	332.5
8	20	380
9	22.5	427.5
10	25	475

TMB Liquid Substrate:

Store TMB Liquid Substrate at 4ºC (light sensitive).

TMB is at working concentration. Bring bottle to ambient temperature, and then pour off desired volume immediately before use. To calculate the volume, multiply the number of plates by 11ml.

Stop Solution:

Store Stop Solution at 4ºC.

Stop Solution is at a working concentration. Bring bottle to ambient temperature, and then pour off desired volume, multiply the number of plates by 11 ml.

Plate Preparation

1. Dilute capture antibody with PBS to a concentration of 1 µg/ml for polyclonal antibody and at least 2 µg/ml for monoclonal antibody. Immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash plates 4 times. Each wash consists of adding 300 µl wash buffer per well, followed by aspiration. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300 µl blocking buffer to each well. Incubate 1 hour at room temperature.
4. Aspirate and wash plate 4 times (as in step 2).

ELISA Protocol

Standard/Sample: Serial dilute standard in accordance with the Certificate of Analysis. Add 100 µl of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

Detection: Wash plate four times. Dilute detection antibody (biotinylated) in accordance with the Certificate of Analysis. Immediately add 100 µl per well. Incubate at room temperature for 2 hours.

Streptavidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate in accordance with the Certificate of Analysis. Add 100 µl per well. Incubate 30 minutes at room temperature. (Dilutions can also be tried at 1:10,000 and 1:40,000, though a dilution of 1:20,000 is most commonly used.)

TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100 µl of substrate solution to each well. Incubate at room temperature for 20 minutes. Add 100 µl of 1M HCl stop solution to each well. Monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 620 nm.

Note: Reliable standard curves are obtained when O.D. readings do not exceed 0.150 units for the zero standard concentrations. Development time and O.D. readings may vary.

Option 2: Preparing Reagents and Materials Yourself

Standard and Antibody

Recombinant Protein[1]
Antigen Affinity Purified Polyclonal or Monoclonal Antibody[1]
Biotinylated Antigen Affinity Purified Polyclonal Antibody[1]

Note: Standard and Antibodies should be reconstituted according to the Certificate of Analysis that accompanies each product.

Recommended Materials

ELISA Microplates (Nunc MaxiSorp Cat. No. 439454 or Corning Cat. No. 3590)
Tween-20 (Sigma Cat. No. P-7949)
BSA (Sigma Cat. No. A-7030)
Streptavidin-HRP Conjugate (Pierce Cat. No. 31032)[1]
TMB Liquid Substrate Solution (KPL Cat. No. 52-00-02)
Dulbecco's PBS [10x] (Gibco BRL Cat. No. 14200-075)
Sealing Film

[1]Included in ELISA Matched Antibody Pair Kits

Recommended Solutions

All solutions should be at ambient temperature prior to use.
PBS: Dilute 10xPBS to 1xPBS, pH 7.20 in sterile water
Wash Buffer: 0.05% Tween-20 in PBS
Block Buffer: 1% BSA in PBS*
Diluent: 0.05% Tween-20, 0.1% BSA in PBS*

**Sterile filter and store 4°C for up to 1 week*

Plate Preparation

1. Dilute capture antibody with PBS to a concentration of 1 µg/ml for polyclonal antibody and at least 2 µg/ml for monoclonal antibody. Immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash plates 4 times. Each wash consists of adding 300 µl wash buffer per well, followed by aspiration. After the last wash invert plate to remove residual buffer and blot on paper towel.
3. Add 300 µl blocking buffer to each well. Incubate 1 hour at room temperature.
4. Aspirate and wash plate 4 times (as in step 2).

ELISA Protocol

Standard/Sample: Serial dilute standard in accordance with the Certificate of Analysis. Add 100 µl of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours.

Detection: Wash plate four times. Dilute detection antibody (biotinylated) in accordance with the Certificate of Analysis. Immediately add 100 µl per well. Incubate at room temperature for 2 hours.

Streptavidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute Streptavidin-HRP conjugate in accordance with the Certificate of Analysis. Add 100 µl per well. Incubate 30 minutes at room temperature. (Dilutions can also be tried at 1:10,000 and 1:40,000, though a dilution of 1:20,000 is most commonly used.)

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