Buffer Preparation for ELISA

10X PBS
NaCl: 80g
KCl: 2g
Na2HPO4: 14.4g
KH2PO4: 2.4g
ddH2O: 800ml
Dissolve well.
Adjust pH to 7.4.
Bring up the volume to 1 L with ddH2O
Sterilize by autoclaving.

1X Wash Buffer
10X PBS: 100ml
ddH2O: 900ml
Tween-20: 50u

1X Blocking Buffer
10X PBS: 10ml
ddH2O: 90ml
BSA: 1g
Dissolve well.

Stop Solution
0.5M H2SO4

ELISA General Protocols

1. Coat 96-well plate with capture antibody overnight at room temperature or 4 ° C for 1-2 days. (Antibody should be coated with the concentration of 1-10µg/ml, 50µl/well in 1X PBS)
2. Wash antibody-coated plate 3X with Wash buffer.
3. Block with 200µl/well of blocking buffer for 30 minutes at RT.
4. Prepare serial dilutions of standards in PBS.
5. Add samples, standards and blank into antibody-coated microtiter plate (50µl/well, duplicates) and incubate for 2 hours at RT.
6. Wash 3X with wash buffer.
7. Add biotinylated Capture antibody and incubate for 1 hour at RT. (Concentration of 1-10µg/ml, 50µl/well in 1X PBS)
8. Wash 5X with Wash buffer.
9. Add Streptavidin-HRP in Wash buffer (50µl/well) and incubate for 45 mins at RT.
10. Wash 5X with Wash buffer.
11. Add TMB substrate (50µl/well) and watch for color change.
12. Stop reaction with Stop solution (50µl/well).
13. Measure OD at 450nm in an ELISA plate reader. (Reference wavelength 620nm)

How Not to Fail a ELISA Experiment?

1. Ensure consistency between wells

Use multiwell plates, multichannel pipettes and plate washers for a more consistent result. Make sure that all pipettes are accurately calibrated on a regular basis. In the initial stage of assay development, test a range of parameters to optimize ELISA conditions.

2. Prevent sample degradation

Protease inhibitors or phosphatase inhibitors can be added into the freshly prepared cell lysis buffer to prevent proteolytic degradation of target proteins. It is important to test all samples in duplicate or triplicate in conjunction with a known standard to ensure the accuracy of results.

3. Optimize coating condition

Coating of antibodies or antigens onto plastic surface is a passive absorption process which depend highly on time, temperature, pH and the concentration of coating agents. Typical coating conditions fall within the range of 1-10 μg/ml in 50-100 μl buffer, incubating overnight at 4 ° C or 1-3 hours at RT. Optimization for each assay need to be performed individually.

4. Choose the right antibodies

Antibodies of high specificity, affinity and avidity need to be used for ELISA assays. Monoclonal antibodies offer better homogeneity by targeting a single epitope while polyclonal antibodies consist of complex antibody pools that target various epitopes. For sandwich ELISA, arigo offers ELISA antibody duos for the optimized performance of ELISA assays.