References

1. Hornbeck P, Winston SE, Fuller SA. Enzyme-Linked Immunosorbent Assays (ELISA). In: Current Protocols in Molecular Biology. 2001: Unit 11.2.
2. Nix B, and Wild D. Data processing. In: Gosling JP, ed. Immunoassays, A Practical Approach. Oxford University Press; 2000: 239-261
3. NCCLS. Evaluation of the Linearity of Quantitative Analytical Methods; Proposed Guideline Second Edition. NCCLS Document EP6-P2 (ISBN 1-56238-446-5), NCCLS, Wayne, Pennsylvania USA, 2001.
4. FDA Guidance for Industry. Bioanalytical Method Validation; May 2001, available for download at www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf.

For More Information

visit **http://advansta.com/products/EIA-Coating-Buffer** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

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10X EIA Coating Buffer

Immunoassay plate coating solution

For Catalog Number

R-03730-D25 10X EIA Coating Buffer, 250 ml

Description

EIA coating buffer can be used with any EIA/ELISA system, including chemiluminescent and colorimetric. Provided as a 10X concentrate.

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10X EIA Coating Buffer

Storage Information

10X EIA Coating Buffer is stable at room temperature for at least one year.

Warnings and Precautions

- 10X EIA Coating Buffer is for research use only.
- Always wear gloves when handling reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when the enclosed protocol is followed by properly trained personnel.

Additional Items Required

- R-03024-D50: AdvanWash™ Washing Solution, 500 mL
- R-03728-E10: AdvanBlock™-EIA Blocking Solution, 1 L

Short Protocol

1. Prepare 1X EIA Coating Buffer by combining 1 part of 10X EIA Coating Buffer with 9 parts of high purity water.
2. Coat the ELISA plate with capture antibody diluted in 1X EIA Coating Buffer and incubate 1h at room temperature (RT). General guidelines for capture antibody: use 0.01-1 µg/well.
3. Wash the plate with 1X AdvanWash™ Washing Solution 4-5 times (200-300 µL/well per wash).
4. Block the plate with 200 µL/well AdvanBlock™-EIA and incubate 1h at RT.
5. Wash the plate with 1X AdvanWash™ Washing Solution 4-5 times (200-300 µL/well per wash).
6. Add the standards and samples (50-100 µL/well diluted in AdvanBlock™-EIA) and incubate 1h at RT.
7. Wash the plate with 1X AdvanWash™ Washing Solution 4-5 times (200-300 µL/well per wash).
8. Add detection antibody diluted in AdvanBlock™-EIA and incubate 1h at RT. General guideline for detection antibody: use 0.1-0.5 µg/mL and 50-100 µL/well.
9. Wash the plate with 1X AdvanWash™ Washing Solution 4-5 times (200-300 µL/well per wash).
10. Add HRP-conjugated antibody directed against the detection antibody diluted 1:10,000 to 1:50,000 in 1X AdvanBlock™-EIA.
11. Wash the plate with 1X AdvanWash™ Washing Solution 4-5 times (200-300 µL/well per wash).
12. Add 100 µL/well substrate (follow manufacturer's recommendations).

Optimization

We recommend a checkerboard titration experiment to define the optimal concentrations for each reagent as previously described.[1] Once the optimal assay conditions are determined, these variables are kept constant from experiment to experiment. A standard curve should be constructed by plotting the known concentration of standards versus signal. All samples, including standards and "unknowns" and their dilutions should be prepared using the same matrix. The dose response curve for many immunoassays tends to have a sigmoidal shape. The best overall fit is often obtained using an algorithm that provides a weighted theoretical model, such as a 4-parameter or 5-parameter logistic curve fit.[2,3] The coefficient of determination (R^2) is a valuable indicator of the overall fit and may be used as one of the criteria in the selection of a curve-fitting method. Overall, the simplest model that defines the concentration-response relationship should be used.[4]