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.

Troubleshooting

If the signal fades quickly, optimize your assay for each reagent as described above. Excess HRP may exhaust the substrate and light emission will decay quickly. It is advisable to reduce the concentration of HRP conjugate.

In general, chemiluminescent ELISAs require 2-10 times less reagents compared to standard colorimetric ELISAs and provide an extended linear range of detection.

For more information

Visit **www.advansta.com/ELISABright** or go directly to the website by scanning the QR code with your favorite barcode scanner app on your smart phone:

Advansta Corporation
2140 Bering Drive | San Jose, CA 95131
Tel: 650.325.1980 | Fax: 650.325.1904
Email: support@advansta.com
www.advansta.com

ELISABright™

Chemiluminescent HRP substrate for ELISA applications

For Catalog Numbers

K-16025-C10	ELISABright, 10 ml sufficient for one (1) 96-well ELISA plate
K-16025-D10	ELISABright, 100 ml sufficient for ten (10) 96-well ELISA plates
K-16025-D25	ELISABright, 250 ml sufficient for twenty-five (25) 96-well ELISA plates

advansta

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Description

ELISABright™ is an enhanced and highly sensitive chemiluminescent HRP substrate optimized for chemiluminescent ELISA applications. This luminol-based substrate demonstrates a wide linear dynamic range and a superior signal-to-noise ratio. ELISABright allows for enhanced detection of low abundance proteins and reduced consumption of antibodies.

Storage

The ELISABright ECL reagents are stable at room temperature for at least one year.

Short Protocol

Step
1. Coat the ELISA plate with diluted capture antibody and incubate 1h at room temperature (RT). General guideline for capture antibody: use 0.01-1 µg/mL and 50-100 µL/well in bicarbonate coating buffer, pH 9.6, composed of 15 mM sodium carbonate and 35 mM sodium bicarbonate.
2. Wash the plate with 1X PBST (PBS + 0.05% Tween 20) four times (200-300 µL/well per wash).
3. Block the plate with 1% BSA-PBST (200 µL/well) and incubate 1h at RT.
4. Wash the plate with 1X PBST four times (200-300 µL/well).
5. Add the standards and samples (50-100 µL/well) and incubate 1h at RT
6. Wash the plate with 1X PBST four times (200 µL/well).
7. Add detection antibody diluted in 1% BSA-PBST and incubate 1h at RT. General guideline for detection antibody: use 0.1-0.5 µg/mL and 50-100 µL/well.
8. Wash the plate with 1X PBST four times (200-300 µL/well).
9. Add HRP-conjugated antibody directed against the detection antibody diluted 1:10,000 to 1:50,000 in 1% BSA-PBST, such as Advansta Goat anti-rabbit (R-05072-500) or Goat anti-mouse (R-05071-500) HRP conjugates.
10. Wash the plate with 1X PBST four times (200-300 µL/well).
11. Mix ELISABright components 1:1 in sufficient amounts to obtain 9.6 mL of substrate solution mixture per each 96-well plate.
12. Use 100 µL of substrate solution (2 kit components mixed 1:1) per well to develop the plate. Measure the signal immediately after addition of substrate.
13. Read the plate on an ELISA microplate reader, such as the 2104 EnVision® (Perkin Elmer), SpectraMax® M5 (Molecular Devices) or similar instrument. Use settings for chemiluminescence detection recommended by the instrument manufacturer.

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 luminescence. 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]