ELISABright™

Chemiluminescent substrate for ELISA applications

Advansta's 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.

ELISA – Enzyme-Linked ImmunoSorbent Assay

An ELISA or Enzyme-Linked ImmunoSorbent Assay is a widely employed biochemical technique used to detect and quantify secreted or intracellular proteins within a variety of sample types including but not limited to sera, cell lysates and plasma.

ELISA methods combine the specificity of antibodies with the sensitivity of enzyme assays by coupling antibodies or antigens to an enzyme, such as horseradish peroxidase (HRP). ELISAs can be used to detect the presence of antigens and analytes, such as proteins, peptides, and hormones, that are recognized by an antibody, or alternatively, to detect antibodies that recognize an antigen. ELISAs are routinely used in scientific research, veterinary medicine, environmental and agricultural applications, and healthcare.

There are three different types of ELISAs: direct, indirect, and capture or "sandwich" assays (Figure 1). All of these assays are performed in a microtiter plate format.

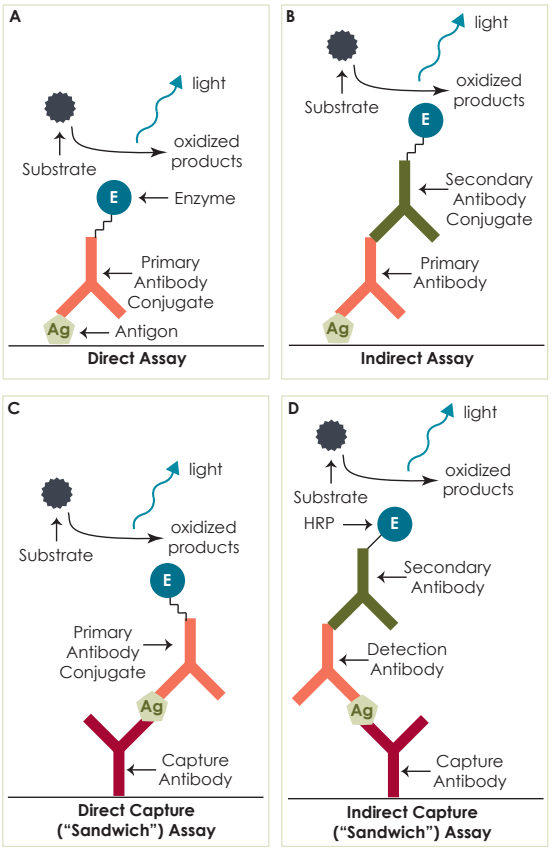

Figure 1. Schematics of direct (A), indirect (B) and capture or "sandwich" (C, D) ELISA. Antigens (Ag) or other analytes can be directly bound to the bottom of the microtiter plate (Figure 1 A and B). After washing and blocking, the antigen is detected by incubating the plate with the antibody conjugated to a reporter molecule – an enzyme such as horseradish peroxidase (HRP). The unbound detecting antibody is washed away and the plate is developed with a solution of peroxidase substrate that emits light as the measure of signal output (Figure 1A). Alternatively, the primary, analyte specific antibody can by unlabeled and a secondary species-specific HRP conjugate can be used (Figure 1B). For sandwich ELISA or capture assays (Figure 1, C and D) the antigens or analytes are bound to the microtiter plate previously coated with a capture antibody. After washing and blocking the captured analyte is detected by incubating the microtiter plate with another analyte-specific antibody containing a reporter molecule. The unbound detecting antibody is washed away and the plate is developed with a solution of peroxidase substrate (Figure 1C). Alternatively, the secondary analyte specific antibody can by unlabeled and a third species-specific HRP conjugate can be used Figure 1D. In all cases the light emission is proportional to the concentration of analyte present in the sample.

Capture assays (Figure 1C and D) have been reported to be 2–5 times more sensitive than those in which the antigen is directly bound to the solid phase (Figure 1A and B). Antibody capture assays are therefore often used for detection of low-abundance analytes.

The sensitivity of ELISA assays can be further enhanced by replacing commonly used colorimetric substrates with a chemiluminescent substrate, such as ELISABright from Advansta for fast detection, high sensitivity, and for superior signal-to-noise ratio.

As demonstrated below (Figures 2 and 3), ELISABright demonstrates a wide linear dynamic range and a signal-to-noise ratio that is at least 6-times greater than that of the competition. ELISABright is ideally suited for assay development as well as for replacement of less-sensitive substrates that require additional incubation time.

Broad linear dynamic range of **ELISA**Bright luminol based substrates

Wide linear dynamic range

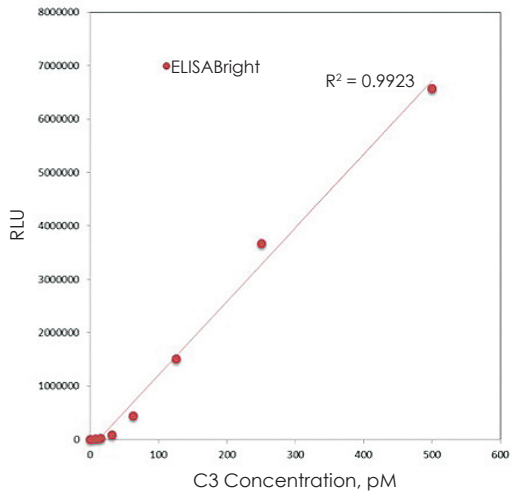

Figure 2. Linear Dynamic Range of ELISABright. ELISABright was used in a sandwich style ELISA (Fig. 1D) to develop duplicate chemiluminescent ELISA experiments. Two-fold serial dilutions of human C3 protein were made in 1% BSA-PBST, spanning the range from 7.81 to 500 pM. 50 µl of each C3 protein solution was added per well, in triplicate, to 96-well EIA strip-well plates (Corning® Costar®) coated with purified anti-C3 antibody, and blocked with 1% BSA-PBST. C3 protein was detected with 50 µl per well of a 0.5 µg/ml solution of a monoclonal anti-huC3a antibody diluted in 1% BSA-PBST followed by a goat anti-mouse-HRP conjugate diluted 1:30,000 in 1% BSA-PBST. Detection was performed according to the ELISABright protocol using 100 µl substrate (components mixed 1:1) per well. The luminescent signal was measured with a Perkin Elmer Envision 2104. ELISABright produces a signal linear with respect to the target protein concentration, with R^2 values approaching 1.

Best signal-to-noise ratio

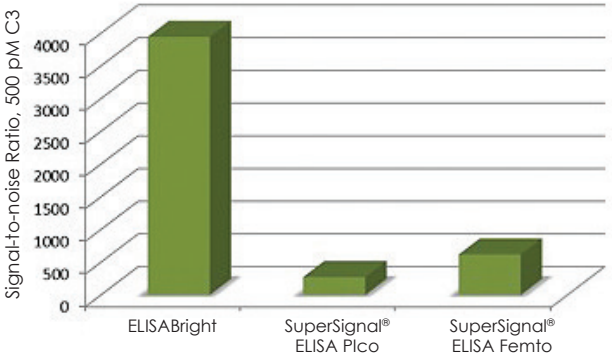

Figure 3. ELISABright provides the highest signal/noise ratio in chemiluminescent ELISA experiments. ELISABright, SuperSignal® ELISA Pico and SuperSignal ELISA Femto substrates (Thermo Fisher) were used to develop replicate wells of a chemiluminescent ELISA as described in detail in Figure 2.

Signal-to-noise ratios for a 500 pM solution of C3 protein were determined for each of the chemiluminescent substrates. ELISABright produced a signal-to-noise ratio over 6 times greater than that of SuperSignal ELISA Femto.

Ordering Information

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

Related Products

R-05071-500	Goat-anti-mouse HRP- conjugated secondary antibody	500 µl
R-05072-500	Goat-anti-rabbit HRP-conjugated secondary antibody	500 µl
R-03024-D50	AdvanWash™ Washing Solution	500 ml
L-07014-100	LucentBlue™ X-ray film, 8 x 10" sheets	100 sheets
K-12045-D20	WesternBright® ECL Western Blotting HRP Substrate (for 2000 cm^2 membrane)	200 ml
K-12049-D50	WesternBright® ECL Spray	500 ml
K-02101-010	Afyon™ SDS-PAGE Sample Preparation Kit	10 rxns

Copyright © 2013-2020 Advansta. All rights reserved. The Advansta logo and WesternBright® are registered trademarks of Advansta Corporation. AdvanWash™, Afyon™, ELISABright™, and LucentBlue™ are trademarks of the Company. All other trademarks, service marks and tradenames appearing in this brochure are the property of their respective owners.

Advansta Corporation
2140 Bering Drive | San Jose, CA 95131
Tel: 650.325.1980 | Fax: 650.325.1904 | Email: sales@advansta.com
Product information: www.advansta.com/products/ELISABright