CYANAGEN

Reagents for Genomics and Proteomics

ELISTAR EtaC Ultra ELISA

Chemiluminescent Substrate for HRP Detection in ELISA

Prod. No. XLSE024

Kit includes:

- Luminol-Enhancer Solution amber bottle
- Peroxide Solution clear bottle

ELISTAR EtaC Ultra ELISA is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

ELISTAR EtaC Ultra ELISA Chemiluminescence HRP Substrate (Product XLSE024)

is an extremely sensitive enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) conjugates in ELISA. Its very intense signal output at 425 nm enables detection of **low femtogram** (10^{-15}) amounts of antigen.

For signal detection a luminometer is required.

GENERAL PRODUCT INFORMATION

- High performance
- Light generation is immediate
- Suitable for high-throughput applications
- As in any ELISA procedure, optimization of antibody, antigen and HRP-conjugate is essential
- **Do not use** sodium azide (NaN_3) as a preservative in buffers as it is a HRP inhibitor.
- To decrease background signal use a detergent. For example, Tween-20 may be added to the blocking reagent at a 0.05% final concentration
- 7-hour Working Solution stability

REQUIRED MATERIALS

1. 96-well *opaque* microplate
2. Capture Antibody in carbonate/bicarbonate buffer, pH 9.4 at 5-10 µg/ml
3. Wash Buffer such as TBS (0.25 M Tris, 0.15 M NaCl; pH 7.2) or PBS (0.1 M phosphate, 0.15 M sodium chloride; pH 7.2)
4. Blocking Buffer containing 0.05% Tween-20.
5. Primary antibody at 0.05-0.1 µg/ml
6. HRP – conjugate at 10-20 ng/ml

PROTOCOL

FOR MICROPLATE DETECTION OF HRP

1. Coat, block and wash microplate wells according to standard ELISA procedures.
2. Prepare ELISTAR EtaC Ultra ELISA Working Solution by mixing equal parts of Luminol and Peroxide Solutions. The Working Solution is stable at least 8 hours at room temperature. DO NOT expose Working Solution to intense light. Store in an amber bottle.
3. Add 100-150 µl of ELISTAR EtaC Ultra ELISA Working Solution to each microplate well.
4. Mix liquid in wells for 1 minute using a microplate stirrer.
5. Use a microplate luminometer to measure relative light units (RLU) at 425 nm, between 1-5 minutes after adding the Working Solution. Longer exposure may result in decreased signal intensity.
6. Note: A test tube luminometer can be used, by increasing the volume of Working Solution as needed.

STORAGE

Upon recept stored at 4°C.
Shipped at ambient temperature
Stability one year

References

1. Kricka, L.J. (2000) *Methods Enzymol.* **305,** 370-390.
2. Heindl, D. and Josel, H.P. (1997) *Non-radioactive Analysis of Biomolecules*, page 258-261. Springer, Berlin.
3. Marzocchi, E., Grilli. S., Della Ciana, L., Prodi, L., Roda, A. and Mirasoli, M., (2008) *Anal. Biochem*., **377**, 189-194.

ELISTAR is manufactured in compliance with our ISO 9001 certified quality management system. ELISTAR is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

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