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A Geno Technology, Inc. (USA) brand name

OptiBlaze ELISA *femto*-HRP

Chemiluminescent Substrate for ELISA

(Cat. #786-302)

INTRODUCTION

OptiBlaze ELISA *femto*-HRP is a two components chemiluminescent detection system for Enzyme-Linked Immunosorbent Assay (ELISA). The chemiluminescent substrate provided in the kit is an improved, ultra sensitive substrate developed for luminometer-based applications, specific for peroxidase labeled antibodies. The kit components are enough for performing 1,000 reactions as per the protocol.

ITEMS SUPPLIED (Cat. #786-302)

Description	Size
OptiBlaze ELISA *femto* Luminol Solution-A	50ml
OptiBlaze ELISA femto Peroxide Solution-B	50ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components at $4^{o}C$.

Note: *The OptiBlaze ELISA femto-HRP Luminol Solution-A is light sensitive and should be protected from direct sunlight or UV sources.*.

ITEMS NEEDED BUT NOT SUPPLIED

Highest purity primary antibody, horseradish peroxidase (HRP) - labeled secondary antibody, Coating Buffer, opaque microwell plate.

NOTE: It is important that opaque microwell plates specifically designed and formulated for ELISA should be used.

PREPARATION BEFORE USE

Allow all reagents to come to room temperature before use.

ASSAY TIPS:

- The experimental conditions recommended below are adequate for most applications, however, variables such as primary and secondary antibody concentration, incubation time, etc. can be modified or adjusted to meet individual assay needs.
- Each of the protocol steps should be evaluated for establishing the optimum conditions that yield maximum sensitivity.
- Use of azide in any of the buffers is not recommended, as it is a known inhibitor of HRP.
- In the absence of luminometer, an x-ray film can be placed over an opaque microplate in a dark room for 1-5 minutes and then the film can be processed by traditional methods.

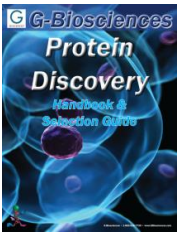
PROTOCOL

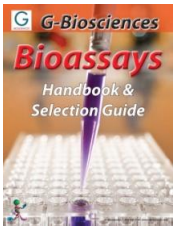
Follow your standard ELISA procedure - incubation, blocking, primary antibody, secondary antibody and washing steps or as detailed below:

1. Apply Antigen diluted in a suitable coating/binding buffer [e.g. 50mM sodium carbonate (pH 9.6) with 20mM Tris-HCl (pH 8.5)] to the ELISA plate wells and incubate at room temperature for 1 hour. After incubation, invert the plate to empty the liquid.
2. Block the well with appropriate blocking buffer (e.g. NAP-Blocker, Cat # 786-190) to each well and incubate the plate for 15-30 minutes. After incubation, empty the blocking buffer from the plate and gently tap out residual liquid.
3. Add specific primary antibody solution (appropriately diluted) to each well and incubate for 1 hour at room temperature. After incubation, empty the plate carefully.
4. Fill each well with 1X TBST (~300µl) and wait for 30 seconds then invert the plate to empty and gently tap out the residual liquid from each well. Repeat the above washing steps 4-5 times.
5. Add 100µl HRP-labeled secondary antibody solution (appropriately diluted) to each well and incubate for 1 hour at room temperature. After incubation, empty the plate and gently tap out the residual liquid.
6. Fill each well with 1X TBST (~300µl) and wait for 30 seconds then invert the plate to empty and tap out the residual liquid from each well. Repeat the above washing steps 4-5 times. Finally add 300µl of 1X TBST into each well and wait for 5 minutes. Tap out the residual wash from each well and is ready to develop.
7. Mix equal parts of OptiBlaze ELISA femto Luminol Solution-A and OptiBlaze ELISA femto Peroxide Solution-B. The working solution is stable up to 24 hrs under ideal conditions in dark. For best results, store the working solution in amber bottle and avoid exposure to light.
8. Add 100µl of the working solution to each well of the ELISA plate. Mix liquid in the wells for 1 minute, using a microplate mixer. Use a luminometer to measure relative light units at 425nm between 1-5 minutes after adding the working substrate solution.
   Note: Longer period between addition of substrate and measuring plate may result in decreased signal intensity. For test tube application, increase working substrate solution volume in the reaction as needed.

RELATED PRODUCTS

Download our Protein Discovery and Assay Development Handbook.





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