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A Geno Technology, Inc. (USA) brand name

OptiBlaze™ ELISA femto-AP

Chemiluminescent Substrate for ELISA

(Cat. # 786-539)

INTRODUCTION

OptiBlaze™ ELISA femto-AP is a one component 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 alkaline phosphatase (AP) labeled antibodies. The supplied reagent is enough for performing 500 reactions as per the protocol.

ITEM(S) SUPPLIED (Cat. # 786-539)

Description	Size
OptiBlaze™ ELISA femto-AP	50ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components at 4°C.

NOTE: *The OptiBlaze™ ELISA femto-AP Solution is light sensitive and should be protected from direct sunlight or UV sources.*

ADDITIONAL ITEMS REQUIRED

Highest purity primary antibody, alkaline phosphatase (AP)-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.
- 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

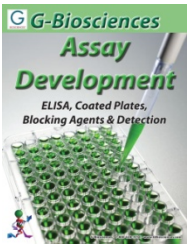
Follow your standard ELISA procedure. A general ELISA protocol is shown below.

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 AP-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. Add 100µl of OptiBlaze™ ELISA femto-AP substrate into each well of the ELISA plate. Mix liquid in the wells for 1 minute, using a microplate mixer.
8. Use a luminometer to measure relative light units at 450nm 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 Assay Development Handbook.



http://info.gbiosciences.com/complete-assay-development-handbook

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Last saved: 8/14/2014 CMH

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