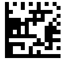

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A Geno Technology, Inc. (USA) brand name

femtoELISA™ HRP Kit

Enzyme-Linked Immunosorbent Assay for Horseradish Peroxidase Labeled Antibodies

(Cat. # 786-110)

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INTRODUCTION

Enzyme-Linked Immunosorbent Assay (ELISA) is one of the most sensitive and powerful techniques for the detection of proteins, chemicals, and drugs (antigens) in biological samples, including serum, blood and urine. The key to an ELISA is the interaction of a known antibody with the antigen of interest, where either antigen or the antibody is immobilized on an ELISA plate micro-well. For the sensitive detection of antigen:antibody complex, secondary antibody labeled with horseradish peroxidase (HRP) is used. G-Biosciences femtoELISA™ HRP kit is supplied with an enhanced blocking agent (NAP-Blocker), an improved, ultra sensitive, stable colorimetric HRP enzyme substrate (tetramethyl benzidine; TMB), and femto-TBST wash buffer. The kit components are enough for performing 1,000 reactions as per protocol.

ITEM(S) SUPPLIED (Cat. # 786-110)

Description	Size
femtoELISA™ HRP Substrate (TMB)	100ml
NAP-Blocker (2X)	250ml
femto-TBST (10X)	2 x 250ml

STORAGE

The kit is shipped at ambient temperature. Upon arrival store the kit components at 4°C. The femtoELISA™ HRP Substrate is light sensitive and should be protected from direct sunlight or UV sources.

ADDITIONAL ITEMS REQUIRED

Highest purity primary antibody, horseradish peroxidase (HRP)-labeled secondary antibody, Coating Buffer, microwell plate designed for immunoassays, 1N HCl or 1N H_2SO_4, microplate reader.

> ***NOTE:*** *It is important that microwell plates specifically designed and formulated for ELISA should be used (polystyrene tissue culture plates are not recommended as they often produce erratic background).*

PREPARATION BEFORE USE

1. Allow all reagents to come to room temperature before use.
2. **10X femto-TBST Dilution**: Dilute the appropriate volume of supplied 10X femto-TBST to 1X with deionized Water.
3. **NAP-Blocker Dilution**: Before use, gently shake the supplied NAP-Blocker bottle to mix it. Use **aseptic techniques** for handling NAP-Blocker. Dilute the appropriate volume of supplied 2X NAP-Blocker 1:2 with 1X femto-TBST.

PROTOCOL

Important Information

1. 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.
2. Each of the protocol steps should be evaluated for establishing the optimum conditions that yield maximum sensitivity.

1. Apply Antigen

Add 100µl Antigen diluted in a suitable coating/binding buffer [phosphate buffered saline or 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 and gently tap out residual liquid.

2. Blocking Step

Add 300µl of diluted [1X] NAP-Blocker to each well and incubate the plate for 15-30 minutes. After incubation, empty the NAP-Blocker from the plate and gently tap out residual liquid.

3. Primary Antibody Reaction

Add 100µl specific primary antibody solution (appropriately diluted in 1X NAP-Blocker) to each well and incubate for 1 hour at room temperature. After incubation, empty the plate carefully and gently tap out the residual liquid.

4. Washing Step-I

Fill each well with 1X femto-TBST (~350µl) 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. Secondary Antibody Reaction

Add 100µl HRP-labeled secondary antibody solution (diluted in 1X NAP-Blocker) to each well and incubate for 1 hour at room temperature. After incubation, empty the plate and gently tap out the residual liquid.

6. Washing Step -II

Fill each well with 1X femto-TBST (~350µl) 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 350µl of 1X femto-TBST into each well and wait for 5 minutes. Tap out the residual wash from each well and plate is ready to develop with femtoELISA™ HRP Substrate.

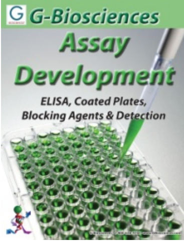
7. Substrate Reaction

After washing step –II, add 100µl of femtoELISA™ HRP Substrate into each well. A soluble blue color develops, which can be read at 370nm or at the 620nm to 650nm range, using femtoELISA™ HRP Substrate as a blank.

For best results, the sample absorbance values should be monitored and read before absorbance values exceed 2.0 OD units. To reduce the intensity of the reaction color, it is recommended to dilute the antibodies or the HRP-conjugates. However, dilution of femtoELISA™ HRP Substrate is not recommended.
In end point assays, the substrate reaction can be stopped, by adding an equal volume (100µl) of 1N HCl or 1N Sulfuric acid to the reaction wells carefully. Addition of acid turns the blue color to yellow and stops the enzymatic reaction.

RELATED PRODUCTS

Download our Assay Development Handbook.



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

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