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A Geno Technology, Inc. (USA) brand name

femto-ELISA-AP Kit

Enzyme-Linked Immunosorbent Assay for alkaline phosphatase labeled antibodies

(Cat. #786-112)

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INTRODUCTION

Enzyme-Linked Immunosorbent Assay (ELISA) is one of the most sensitive and powerful techniques for detecting 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 the antigen or antibody is immobilized on an ELISA plate micro-well. For sensitive detection of antigen-antibody complex, secondary antibody labeled with alkaline phosphatase (AP) is used. G-Biosciences femto-ELISA-AP kit is supplied with an enhanced blocking agent (NAP-Blocker), an improved, ultra sensitive, stable colorimetric alkaline phosphatase substrate (pNPP; p-Nitrophenyl phosphate), and femto-TBST wash buffer. The kit components are enough for performing 1,000 reactions as per the protocol.

ITEM(S) SUPPLIED (Cat. # 786-112)

Description	Size
femto-ELISA-AP Substrate (pNPP; p-Nitrophenyl phosphate), Cat # 786-113	1 x 100ml
NAP-Blocker (2X)	1 x 250ml
femto-TBST (10X), Cat # 786-161	2 x 250ml

STORAGE

The kit is shipped at ambient temperature. Upon arrival store the kit components at 4°C. The femto-ELISA-AP Substrate 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/binding buffer, microwell plate designed for immunoassays, microplate reader, multichannel pipettor etc.

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 DI 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

I. I.The experimental condition recommended in this protocol 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.

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

1. Apply Antigen to each well with suitable Coating Buffer

Add 100µl Antigen, diluted in a suitable coating buffer [e.g. phosphate buffered saline or 50mM Sodium carbonate (pH9.6) 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 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 the residual liquid.

3. Primary Antibody Reaction

Add 100µl specific primary antibody solution (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 1

Fill each reaction well with 1X femto-TBST (~350µl) and wait for 30 seconds then invert the plate to empty. Gently tap out the residual liquid from each well. Repeat the above washing steps 4-5 times.

4. Secondary Antibody Reaction

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

5. Washing Step 2

Fill each reaction well with 1X femto-TBST (~350µl) and wait for 30 seconds then invert the plate to empty. Tap out the residual liquid from each well. Repeat the washing steps 4-5 times as above. Finally add 350�l 1X femto-TBST in each well and wait for 5 minutes. Tap out the residual wash from each well.

6. Substrate Reaction

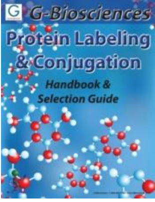
After washing step –II, add 100µl femto-ELISA-AP Substrate into each well. A soluble yellow color develops, which can be read at 405-410nm ranges, using femto-ELISA-AP Substrate as blank.

Fore best results, 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 conjugates. However, dilution of femto-ELISA-AP Substrate is not recommended.

In end point assays, the substrate reaction can be stopped, by adding 50µl of 3N Sodium hydroxide (NaOH) carefully to the reaction wells.

RELATED PRODUCTS

Download our Assay Development Handbook.



http://info2.gbiosciences.com/complete-assay-development-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

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