femto-ELISA-AP Kit

Enzyme-Linked Immunosorbent Assay for alkaline phosphatase labeled antibodies

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 SUPPLIED	**Cat # 786-112**
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

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.

ITEMS NEEDED BUT NOT SUPPLIED

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).

PROTOCOL

Preparation Before Use

A. Allow all reagents to come to room temperature before use.

B. 10X *femto*-TBST Dilution: Dilute the appropriate volume of supplied 10X *femto*-TBST to 1X with DI Water.

C. 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.

Assay Tips:

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

Protocol Steps:

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 - I
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 - II
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 350µl 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

1. ***AP-Labeled Secondary Antibodies***

Cat. #	***Description***	***Size***
786-R43	*Goat ant-Mouse IgG H&L-AP*	*1ml*
786-R44	*Goat anti-Rabbit IgG H&L-AP*	*1ml*
786-R45	*Goat anti-Rat IgG H&L-AP*	*1ml*
786-R46	*Goat anti-Human IgG H&L-AP*	*1ml*
786-R47	*Rabbit anti-Goat IgG H&L-AP*	*1ml*
786-R49	*Rabbit anti-Human IgG H&L-AP*	*1ml*

2. Protein dotMETRIC™ 1µl Protein Assay (Cat. # 786-20)
Apply 1µl of the protein solution to the test strip and develop in 8 minutes. A circular protein spot is produced. The diameter of the protein spot is proportional to protein concentration. By measuring the diameter of protein spots with the dotMETRIC™ gauge, supplied with each kit, you can easily determine protein concentration. (Patents Pending)

3. Non-Interfering (NI) Protein Assay™ (Cat. # 786-005)
NI-Protein Assay™ is not affected by interfering agents commonly present in protein solutions, including reducing agents such as 2ME, DTT, detergents, amines, EDTA, Salts, sugars etc. NI-Protein Assay™ also shows no protein-to-protein variation. The assay is based on removal of interfering agents, prior to assay by a single step protocol. Sensitivity as low as 0.5ug/assay. Assay time is 15-20 minutes.

Note: For other related products, please visit our web site at **www.GBiosciences.com**