

Sandwich ELISA Assay Protocol for Matched Antibody Pair

!! PLEASE READ BEFORE USE !!

- ◆ **Please centrifuge before opening the vials.**
- ◆ **The test protocol is a guideline, user need to determine their optimal experimental condition for best performance.**

SECTION 1 – Reagents

1.1 Matched Antibody pair

Abnova's matched antibody pair (Catalog # format: Hxxxxxxx-APxx) contain a pair of capture antibody and detection antibody for detecting specific protein target. It has different combinations depending on the antibody contents, please see Table 1 for detail.

1.2 Secondary Reagent

Please choose one of the following secondary reagents according to the antibody pair combinations, please see Table 1 for detail.

- **Streptavidin-HRP:** We recommended Peroxidase Streptavidin from Jackson ImmunoResearch Laboratories, Inc (Cat #: 016-030-084)
- **Goat anti-mouse IgG, HRP conjugated:** We recommended Peroxidase AffiniPure Goat Anti-Mouse IgG from Jackson ImmunoResearch Laboratories, Inc (Cat #: 115-035-062)
- **Goat Anti-Rabbit IgG, Peroxidase Conjugated:** We recommended Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated from PIERCE (Cat #: 31460)

1.3 Protein Standard

There are two types of antigen, either recombinant protein or Mammalian Overexpression Lysate is used as protein standard to establish the standard curve, please see the product page on Abnova website for the identity of protein standard use for each matched antibody pair.

1.4 Coating Buffer, 1xPBS

137 mM NaCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 2.7 mM KCl, pH 7.4

1.5 PBST

1xPBS with 0.2% Tween-20

1.6 Blocking Buffer

5% non-fat milk in PBST

1.7 Diluent

2% non-fat milk in PBST

1.8 Substrate Buffer

Dissolve 2.6 g Citric acid and 6.9 g Na₂HPO₄ in ddH₂O to 500 ml, adjust pH to 5.0.

1.9 Substrate

Ophenylenediamine (OPD), 0.4 mg/ml in **Substrate Buffer**, then add one thousandth volume of 30% H₂O₂ (e.g. Add 20 µl 30% H₂O₂ into 20 ml OPD substrate solution). Reagent must be freshly prepared each time before application.

1.10 Stop Solution

2 M H₂SO₄ in ddH₂O

Table 1, Different matched antibody pair combinations and the recommended dilution^b (or concentration) to use in ELISA assay.

Catalog # format ^a	Hxxxxxxx-AP1x	Hxxxxxxx-AP2x		Hxxxxxxx-AP3x	Hxxxxxxx-AP4x
Protein standard	Recombinant Protein	Recombinant Protein		Recombinant Protein	Recombinant Protein
Capture Ab	Rabbit MaxPab® affinity purified pAb	Rabbit MaxPab® affinity purified pAb		Rabbit MaxPab® affinity purified pAb	Mouse mAb
dilution	2.5 µg/ml	2.5 µg/ml	2.5 µg/ml	2.5 µg/ml	2.5 µg/ml
Detection Ab	Mouse mAb	Mouse pAb	Mouse purified pAb	Biotinylated rabbit affinity purified pAb	Biotinylated mouse mAb
dilution	0.5 µg/ml	1/1000x	0.5 µg/ml	0.5 µg/ml	1 µg/ml
Secondary Reagent	Goat anti-mouse IgG, HRP conjugated			Streptavidin-HRP	Streptavidin-HRP
dilution	1/2000x in 1xPBS with 0.05% Tween-20 and 2% skim milk			1/5000x in 1xPBS	1/5000x in 1xPBS

Catalog # format ^a	Hxxxxxxx-AP5x	Hxxxxxxx-AP6x	Hxxxxxxx-AP7x
Protein standard	Mammalian Overexpression Lysate	Mammalian Overexpression Lysate	Recombinant Protein
Capture Ab	Mouse mAb	Mouse mAb	Rabbit purified pAb
dilution	2.5 µg/ml	2.5 µg/ml	2.5 µg/ml
Detection Ab	Rabbit purified pAb	Rabbit affinity purified pAb	Mouse mAb
dilution	1 µg/ml	1 µg/ml	1 µg/ml
Secondary Reagent	Goat Anti-Rabbit IgG, Peroxidase Conjugated	Goat Anti-Rabbit IgG, Peroxidase Conjugated	Goat anti-mouse IgG, HRP conjugated
dilution	1/2500x in 1xPBS with 0.2% BSA	1/2500x in 1xPBS with 0.2% BSA	1/2000x in 1xPBS with 0.05% Tween-20 and 2% skim milk

Note:

^a "x" means a digit number.

^b The information provide in this table shall be used only as a guide, each investigator should determine the optimal experimental condition for their own specific application.

SECTION 2 - Preparation of Reagents

2.1 Preparation of the Capture Ab and 96-well plate

- **Coating:** Apply 100 μ l of **Capture Ab** to each well (96-well plate)
 - ※Please refer to **Table 1** for working concentration of **Capture Ab** (dilute in **Coating Buffer**).
- **Blocking:** Apply 200 μ l of **Blocking Buffer** to each well.

2.2 Preparation of Standard

- **Recombinant protein:** uses series concentrations of 100, 30, 10, 3, 1, 0.3, 0.1, and 0.03 ng/ml to establish the standard curve.
- **Mammalian Overexpression Lysate:** uses series dilutions of 3x, 9x, 27x, 81x, 243x, and 729x fold to establish the standard curve.
 - ※ Dilute the protein standard in **Diluent**.

2.3 Preparation of Detection Ab & Secondary Reagent

Please refer to **Table 1** for the working dilution (or concentration) of the reagents.

※Dilute the **Detection Ab** in **Diluent**.

SECTION 3 - Assay Protocol

- 3.1 **Coating:** Apply 100 μ l of diluted **Capture Ab** in the well (96-well plate). Cover the plate with an adhesive plastic and incubate at 4°C overnight.
- 3.2 Wash the plate twice with **PBST**. The solutions or washes are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.
- 3.3 Block the coated wells by adding 200 μ l **Blocking Buffer**. Cover the plate with an adhesive plastic and incubate for at least 2 hours at room temperature or overnight at 4°C.
- 3.4 Wash the plate twice with **PBST**.
- 3.5 Add 100 μ l of **Protein Standard**/samples diluted at the adequate concentration (please see **SECTION 2.2** for preparation of **Protein Standard**) in **Diluents**. Cover the plate with an adhesive plastic and incubate for 2 hours at room temperature. This incubation time may require optimization.
- 3.6 Wash the plate three times with **PBST**.
- 3.7 Apply 100 μ l of **Detection Ab** in **Diluents**. Cover the plate with an adhesive plastic and incubate for 2 hours at room temperature.
- 3.8 Wash the plate three times with **PBST**.
- 3.9 Apply 100 μ l of **Secondary Reagent** at adequate concentration. Cover the plate with an adhesive plastic and incubate for one hour at room temperature. This incubation time may require optimization.
- 3.10 Wash the plate four times with **PBST**.

3.11 Apply 150 μ l OPD substrate solution into each well. After adequate color development, read the absorbance at 450 nm with an ELISA plate reader.

3.12 Alternatively, add 100 μ l of **stop solution** to the wells and read the absorbance (OD490) of each well with a plate reader.