Betatrophin (139-198) (Human)

Western Blot Kit

PROTOCOL

(Catalog #WBK-051-55)

PHOENIX PHARMACEUTICALS, INC.

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CAUTION:

Investigational device. Limited by law to investigational use.

For research use only. Not for use in diagnostic procedures.

KIT CONTENTS

1. Betatrophin (139-198) (Human) Purified IgG Antibody (*1 vial, 50* μg)
2. Standard Betatrophin (139-198) Peptide (*1 vial, 10* μg)
3. Goat anti-rabbit IgG whole antibody HRP conjugate (*1 vial, 50 μl*)
4. 10 x TBST pH 7.6 (*2 bottles, 100 ml*)
5. 10 x TBS pH 7.6 (*1 bottle, 50 ml*)
6. 10 x Blocking Buffer and Antibody Diluent (*1 bottle, 50ml*)
7. Detection Reagent A (*1 bottle, 30ml*)
8. Detection Reagent B (*1 bottle, 30ml*)
9. Protocol Booklet

(Note: This kit supplies sufficient reagents to perform 5 blots.)

STORAGE

1. The primary antibody, blocking buffer, and antibody diluent should be stored at -20° C.
2. Secondary antibody should be stored at 2 ~ 8°C. **DO NOT FREEZE!**
3. Detection reagent A and B are light sensitive. They are stable for 6 months at 2 ~ 8°C in the dark. Keep bottles closed to prevent microbial or dust contamination.

Phoenix Pharmaceuticals, Inc. guarantees that its products conform to the information contained in general publication. The purchaser must determine the suitability of the product for their particular needs and establish optimum sample concentrations.

INTRODUCTION

Phoenix's Western Blot Kit is designed for the rapid and sensitive immuno-enzymatic detection of specific peptides immobilized on nitrocellulose or polyvinylidene fluoride (PVDF) membranes, whether transferring from SDS-PAGE / Tricine-PAGE gels after electrophoresis (western blot) or bound directly from solution (dot blot). The purified primary antibody (IgG) used for this assay was raised against a synthetic peptide. A highly purified standard peptide is included with the kit to be used as a positive control or to pre-block the primary antibody. All reagents necessary to produce fast and sensitive results on nitrocellulose or PVDF membrane are included. This kit enables the detection of antigen levels of 500ng or lower.

GENERAL DESIGN OF THIS KIT

Phoenix Pharmaceuticals' Western Blot Kit is based on the enzyme-linked immuno-detection of antigen-specific antibody (supplied with the kit) using anti-IgG secondary antibodies conjugated to horseradish peroxidase (HRP), which reacts with a chemiluminescent substrate in the presence of a chemical enhancer. This produces a light signal that can be captured by short exposure to a blue-light sensitive autoradiography film or on the Molecular Imager system. This kit brings a new level of convenience and reliability to a non-isotopic procedure while providing a fast, highly sensitive detection for membrane bound peptides / proteins.

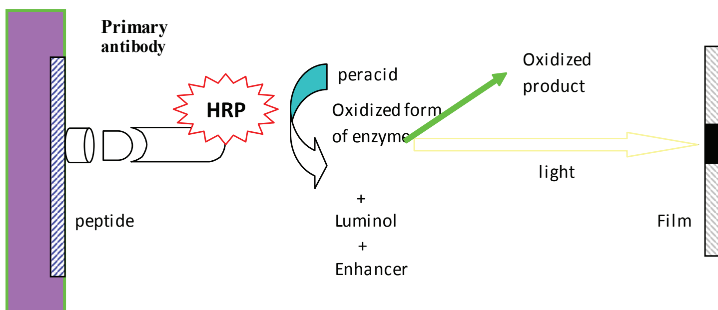

Figure 1. Diagram of the molecular interactions used in this kit

Note: Read this protocol in its entirety before starting the assay. Each kit contains reagents sufficient for 5 Western Blots.

ASSAY PROTOCOL

Blocking of membrane (blot)

To saturate nonspecific protein binding sites, incubate the membrane for 30 minutes in blocking buffer/antibody diluent (TBST containing 1% blot-qualified BSA) for nitrocellulose membranes; incubate 60 minutes for PVDF membranes.

Primary Antibody Binding

1. To bind primary antibody, replace the blocking solution (which can be re-used several times) with blocking buffer/antibody diluent containing appropriate dilution of primary antibody (recommended primary antibody dilution = 1:1000 ~ 2000). Incubate the blot for 30 ~ 60 minutes with gentle agitation at room temperature (or overnight at 2 ~ 8 °C).
2. To remove unbound antibody, wash the membrane three times with TBST for 5 ~ 10 minutes each.

Secondary Antibody Binding

1. Incubate blot with blocking buffer/antibody diluent containing the appropriate dilution of anti-rabbit IgG- HRP conjugate for 30 minutes with gentle agitation. We recommend that the secondary antibody dilution be in the range of 1:3000 ~ 6000.
2. Wash the blot with TBST three times for 10 minutes each to remove unbound secondary antibody.
3. Briefly rinse twice with TBS to remove Tween 20 from the blot surface.

ECL developing

1. Mix equal volumes of detection reagent A and detection reagent B to cover the membranes (based on 0.125 ml / cm2 membrane).

2. Drain the excess buffer from the washed blot (membrane) and place it on a piece of Saran Wrap or a glass plate with the protein or peptide side facing up. Add mixed detection solution to the peptide or protein side of the membrane. Make sure the entire surface of the membrane is covered with the mixed detection solution. Incubate for exactly 5 minute without agitation. Drain off excess detection solution and wrap membranes in SaranWrap. Using gloved fingers, smooth out all air pockets on the membrane surface by pressing gently.
3. Place the blot with the peptide or protein face up, in the film cassette. Work as quickly as possible to minimize the delay between incubating the blot in the mixed detection solution and exposing it to the film. In a dark room, place a sheet of blue light sensitive film on top of the blot, close the cassette and expose for 1 minute according to signal strength. The second exposure may vary from 30 seconds to 7 minutes depending on target signal and background. Perform this step in a dark room using a red safe light..

SPECIAL NOTES:

1. The method of “sample preparation” for blood plasma or cells lysate is important in order to obtained a good results of betatrophin detection. Usually, diluting the samples at least 1:2 with SDS-PAGE sample buffer and to incubate at 37 ˚C (do not over 60˚C) for 20 min is necessary in order to prevent the aggregation of betatrophin proteins becoming a higher Mw molecule.
2. The sensitivity of Western blotting is dependent, in part, on the efficiency of transfer from polyacrylamide gel onto the membrane. In general thinner and lower percentage gels give greater transfer efficiency and thus higher Western blotting sensitivity.
3. The serum abumin protein in the sample may interference with the detection of betatrophin on Western Blot. However, without BSA removal, circulation betatrophin usually can be detected by the betatrophin (139-198) (Human) Purified IgG antibody when applying 1 microliter of serum or plasma per lane in the SDS-PAGE. However, if you need to load more amounts (e.g. > 3 microliter) of serum or plasma per lane into the SDS-PAGE well, it is recommended to remove the containing albumin protein from your samples before it has been the loaded into SDS-PAGE gel.

FOR RESEARCH USE ONLY
NOT FOR USE IN
DIAGNOSTIC PROCEDURES

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