BACKGROUND

Factor VIII is a glycoprotein cofactor that serves as a critical component in the blood coagulation pathway. Insufficient expression levels or expression of nonfunctional Factor VIII results in hemophilia A, a common severe hereditary bleeding disorder. In the liver, the main site of Factor VIII synthesis, the mature polypeptide chain of 2,332 amino acids is secreted into the lumen of the endoplasmic reticulum, where it interacts with various chaperone proteins, including calreticulin, Calnexin and IgG-binding protein. From the lumen, a portion of Factor VIII translocates to the Golgi and undergoes activation via proteolysis of both the heavy and light chain portions of the protein into three fragments. Finally, proteolysis of activated Factor VIII by Factor Xa, Protein C or Thrombin results in inactivation of Factor VIII. Survival of Factor VIII in the bloodstream requires binding to von Willebrand factor (VWF) at both the amino- and carboxy-termini of the light chain. Point mutations occurring in those binding domains as well as at other active sites of Factor VIII likely underly 90-95% of disease cases.

REFERENCES


CHROMOSOMAL LOCATION

Genetic locus: F8 (human) mapping to Xq28; F8 (mouse) mapping to X A7.3.

SOURCE

Factor VIII (R8B12) is a mouse monoclonal antibody raised against Factor VIII of human origin, with epitope mapping to the A2 domain residues 497-510 and 584-593.

PRODUCT

Each vial contains 100 µg IgG κ kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

APPLICATIONS

Factor VIII (R8B12) is recommended for detection of Factor VIII of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) and immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)].


Molecular Weight of Factor VIII heavy chain: 200 kDa.

Molecular Weight of Factor VIII light chain: 80 kDa.

Molecular Weight of Factor VIII cleaved fragments: 50/43/73 kDa.

Positive Controls: human kidney extract: sc-363764, Jurkat whole cell lysate: sc-2204 or Hep G2 cell lysate: sc-2227.

RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended:

1) Western Blotting: use m-IgG κ B-P HRP: sc-516102 or m-IgG κ B-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml).

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

CONJUGATES

See Factor VIII light chain (RFFVII C/5): sc-59512 for Factor VIII light chain antibody conjugates, including AC, HRP, FITC, PE, Alexa Fluor® 488 and Alexa Fluor® 647.