Product datasheet

Factor VIIIa Activity Assay Kit (Fluorometric) ab204696

Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Factor VIIIa Activity Assay Kit (Fluorometric)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection method</td>
<td>Fluorescent</td>
</tr>
<tr>
<td>Sample type</td>
<td>Serum, Plasma, Purified protein</td>
</tr>
<tr>
<td>Assay type</td>
<td>Enzyme activity</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1 ng/well</td>
</tr>
<tr>
<td>Product overview</td>
<td>Factor VIIIa Activity Assay Kit (Fluorometric) is based on the ability of FVIIIa to generate FXa. The generated FXa proteolytically cleaves a synthetic substrate and releases a fluorophore, AMC, which can be easily quantified by fluorescence microplate reader. The assay is simple, rapid and can detect activity as low as 1 ng of FVIIIa in a variety of samples.</td>
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</tbody>
</table>

Notes

The coagulation Factor VIII (anti-hemophilic factor, AHF) is a vital blood-clotting protein. Factor VIII circulates in the bloodstream as an inactive protein, bound to a large multimeric glycoprotein called von Willebrand factor (VWF). Upon an injury to the blood vessel, VWF dissociates from FVIII and releases the active form of FVIII (FVIIIa). In the presence of calcium ions and negatively charged membrane phospholipids, activated factor VIII (FVIIIa) then binds to the activated Factor IX (FIXa) and proteolytically activates factor X (FX) to factor Xa (FXa).

Platform

Microplate reader

Properties

Storage instructions

Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme Mix I</td>
<td>1 vial</td>
</tr>
<tr>
<td>Enzyme Mix II</td>
<td>1 vial</td>
</tr>
<tr>
<td>FVIIIa Assay Buffer</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>FVIIIa Enzyme Standard</td>
<td>1 x 2.6µg</td>
</tr>
<tr>
<td>FXa Substrate-AMC</td>
<td>1 x 200µl</td>
</tr>
</tbody>
</table>
Function

Factor VIII, along with calcium and phospholipid, acts as a cofactor for factor IXa when it converts factor X to the activated form, factor Xa.

Involvement in disease

Defects in F8 are the cause of hemophilia A (HEMA) [MIM:306700]. A disorder of blood coagulation characterized by a permanent tendency to hemorrhage. About 50% of patients have severe hemophilia resulting in frequent spontaneous bleeding into joints, muscles and internal organs. Less severe forms are characterized by bleeding after trauma or surgery. Note=Of particular interest for the understanding of the function of F8 is the category of CRM (cross-reacting material) positive patients (approximately 5%) that have considerable amount of F8 in their plasma (at least 30% of normal), but the protein is non-functional; i.e., the F8 activity is much less than the plasma protein level. CRM-reduced is another category of patients in which the F8C antigen and activity are reduced to approximately the same level. Most mutations are CRM negative, and probably affect the folding and stability of the protein.

Sequence similarities

Belongs to the multicopper oxidase family.
Contains 3 F5/8 type A domains.
Contains 2 F5/8 type C domains.
Contains 6 plastocyanin-like domains.

Domain

Domain F5/8 type C 2 is responsible for phospholipid-binding and essential for factor VIII activity.

Post-translational modifications

Sulfation on Tyr-1699 is essential for binding vWF.

Cellular localization

Secreted > extracellular space.

Images

Typical Factor VIIIa Standard Curve using Factor VIIIa Activity Assay Kit (Fluorometric) (ab204696).
Factor VIIIa activity was measured in serum samples in the presence and absence of the master mix. S: Substrate.

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