IMMBIOMED

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# SPECTROZYME® FIXa Chromogenic substrate

## Product No. ADG299

### Description

Highly sensitive chromogenic substrate for factor IXa. The sensitivity of this substrate is significantly enhanced in the presence of alcohols, especially ethylene glycol.

### Chemistry

**Formula:** $CH_3SO_2$-(D)-CHG-Gly-Arg-pNA·AcOH
**Chemical name:** Methylsulfonyl-D-cyclohexylglycyl- glycyl-arginine-paranitroanilide monoacetate salt

**MW:** 628.7 Dalton

**Solubility:** up to 20 mM in water

**Biochemical Characteristics:**
$k_{cat}$ = 4.4/sec $K_M$ = 1.3 mM
$k_{cat}/K_M$= 3.38 l/mM·s (determined in the presence of 33% ethylene glycol)

### Intendet use

Determination of Factor IXa in concentrates or other blood products according to Ref 1.

**Conditions:** The test temperature can be selected but should be kept constant during the assay. All reagents should be kept at the test temperature prior to use. Do not work with chilled reagents directly from the refrigerator. For the kinetic version 37°C may be used, especially when a thermo-stated cell holder is available.

**Automation:** The assay can be either performed on a spectrophotometer or microtiter plate reader at 405 nm. Kinetic or endpoint versions are possible. An adaptation on fully automated chemistry analyzers at 405 nm may be possible but has not been tested.

**Buffer:** 50 mM Tris, pH 7.4, 100 mM NaCl, 5 mM $CaCl_2$, 40 % (vol/ vol) ethylene glycol.

**Notes:** The sensitivity of this substrate for factor IXa is significantly increased in the presence of 33% ethylene glycol.
All volumes of the described pipetting scheme may be adapted for assay in regular cuvettes. An example is given below.
Interference by turbidity or from colored samples in the endpoint assay can lead to falsely elevated results. This can be prevented by running a sample blank as follows:
Pipette a sample blank in the following sequence: Acetic Acid/„Stop reagent“ - Buffer sample - Substrate

### Microtiter plate format

0.200 ml buffer
0.025 ml Spectrozyme® FIXa (10 mM)
0.020 ml Sample (factor IXaβ, 2 µM)
⇒ Determination of optical density at 405 nm
0.025 ml Acetic acid (50 %) to stop the reaction after 5-10 minutes

### Spectrophotometer format

0.800 ml buffer
0.100 ml Spectrozyme FIXa (10 mM)
0.080 ml Sample (factor IXaβ, 2 µM)
⇒ Determination of optical density at 405 nm
0.100 ml Acetic acid (50 %) to stop the reaction after 5-10 minutes

Calculate the activity of factor IXa according to:
F IXa activity = (OD sample – OD sample blank)

### Warnings and Precautions

**Contains:** para-nitroanilide acetate salt

**Hazard pictograms**

**Signal word** Warning

**Hazard statements**
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

**Precautionary statements**
P261 Avoid breathing dust.
P280 - Wear protective gloves.
P312 - Call a POISON CENTER/doctor if you feel unwell.
P332 + P313 - If skin irritation occurs: Get medical advice/attention.
P337 + P313 - If eye irritation persists: Get medical advice/attention.
P501 - Dispose of contents/container in accordance with local/regional/ national/international regulations.

### Presentation

Amber glass vial containing 10 µmoles of lyophilized substrate.

### Reconstitution

Reconstitute with filtered deionized water to create a 10 mM solution. Shake gently before use.

### Storage

May be used by the expiry date given on the label when stored unopened, protected from moisture, in the dark, 2-8°C. Avoid contamination of the reagents by microorganisms.

### References

1. Dramatic enhancement of the catalytic activity of coagulation factor IXa by alcohols. Stürzebecher J et al., FEBS Lett 1997; 412:295-300
2. Determination of activated factor IX in factor IX concentrates with a chromogenic substrate. Prasa D and Stuerzebecher J. Throm Res; 92:99-102.

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