SPECTROFLUOR™ FIXa

**REF** 299F and 299FL

**Description**
A fluorogenic substrate for the amidolytic assay of factor IXa activity in purified preparations.

**Formula:** \( \text{CH}_3\text{SO}_2\text{-D-CHG-Gly-Arg-AMC-AcOH} \)

**Molecular Weight:** 665.8

**Chemical Name:** methylsulfonyl-D-cyclohexylglycyl-glycyl-arginine-7-amino-4-methylcoumarin acetate salt

**Composition:** Enzymatically digestible substrate colyophilized with glycine excipient

**Purity:** \( \leq 0.5\% \) free AMC

**Solubility:**
- > 10 mM in distilled/deionized water
- > 1.0 mM in 0.05 M Tris Buffer, pH 7.4

**Optical Characteristics:**
- Absorption Maximum Wavelength, \( \lambda_{\text{Abs}} \): 342 nm
- Emission Maximum Wavelength, \( \lambda_{\text{Em}} \): 440 nm

**Assay Conditions/Substrate Kinetics**
Enzyme activity is determined by measuring the increase in fluorescence of the free fluorophore (AMC) generated, in comparison to the original substrate, per unit time at \( \lambda_{440} \) nm. At excess substrate concentration, the rate of fluorescence increase due to the amount of fluorophore released is linearly related to enzyme concentration. Measurement can be made either through acid quenching of the reaction (end-point method), or through use of a kinetic recording fluorometer (initial-rate method).

Under the following reaction conditions, the following substrate kinetics were found,

**Substrate:** 25 \( \mu \)L of SPECTROFLUOR™ FIXa at a 10 mM stock concentration

**Buffer:** 200 \( \mu \)L of 50 mM Tris, 100 mM NaCl, 5 mM CaCl\(_2\), 40% ethylene glycol, pH 7.4

**Enzyme:** 20 \( \mu \)L of human Factor IXa (REF 449B) at a 19.4 \( \mu \)g/mL concentration

Incubate for 3 minutes at 25°C (room temperature).

**Kinetics:**
- \( K_m \): 0.23 mM
- \( V_{\text{max}} \): 28.1 \( \mu \)mole/min

**Presentation**

| REF 299F | Amber glass vial containing 10 \( \mu \)moles of lyophilized substrate. |
| REF 299FL | Amber glass vial containing 50 \( \mu \)moles of lyophilized substrate. |

**Reconstitution**

| REF 299F | Dissolve substrate with 1 mL of filtered deionized water to generate a 10 mM stock solution. |
| REF 299FL | Dissolve substrate with 5 mL of filtered deionized water to generate 10 mM stock solution. |

**Storage and Stability**
Lyophilized substrate may be stored in the dark at 2° - 8°C up to the expiration date stated on the vial. Protect from moisture by allowing vial to reach room temperature prior to opening.

Reconstituted substrate may be stored in the dark for 1 week at room temperature, 2 months at 2° - 8°C, or for up to 6 months frozen at –20°C (Aliquot and freeze. Do not submit to freeze-thaw cycles).

**Warnings and Precautions**

| CONT | 7-amino-4-methylcoumarin |

For Research Use Only

**Warning**

<table>
<thead>
<tr>
<th>Hazard Statements:</th>
</tr>
</thead>
<tbody>
<tr>
<td>H315 Causes skin irritation.</td>
</tr>
<tr>
<td>H319 Causes serious eye irritation</td>
</tr>
<tr>
<td>H335 May cause respiratory irritation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precautionary Statements:</th>
</tr>
</thead>
<tbody>
<tr>
<td>P261 Avoid breathing dust.</td>
</tr>
<tr>
<td>P264 Wash thoroughly after handling.</td>
</tr>
<tr>
<td>P280 Wear protective gloves/eye protection</td>
</tr>
<tr>
<td>P337 + P313 If eye irritation persists: Get medical advice/attention</td>
</tr>
<tr>
<td>P403 + P233 Store in a well-ventilated place. Keep container tightly closed.</td>
</tr>
</tbody>
</table>

**Related Products**

REF 229, SPECTROZYME® FIXa, a chromogenic substrate
REF 449B, human Factor IXa
Definition of Symbols

- Consult instructions for use
- Manufacturer
- Refer to Safety Data Sheet
- Contains…
- Temperature Limitation
- Lot Number
- Expiration Date
- Catalog Number