Pentapharm Ltd. offers a broad spectrum of fluorogenic peptide substrates for research, in-process and quality control, employing the fluorogenic group AMC (7-Amino-4-methylcoumarin).

Optical characteristics of AMC:
Absorption maximum wavelength: \( \lambda_{\text{Abs.}}: 342 \text{ nm} \)
Emission maximum wavelength: \( \lambda_{\text{Em.}}: 440 \text{ nm} \)

**Pefafluor FXa**

**Application:** Highly sensitive fluorogenic peptide substrate for factor Xa.

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

\( k_{\text{cat}}: \) 162.0 s\(^{-1}\)

\( K_M: \) 0.22 mM

**Solubility:** Up to 10 mM in dest. H\(_2\)O

**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.

Shipment of product does not require cooling during the time of transportation.

**Assay:** Suggested protocol for the determination of factor Xa activity:

- 0.900 ml 50 mM Tris-HCl pH 7.4, 100 mM NaCl, 0.5% HSA
- 0.100 ml Factor Xa (KORDIA, 11.8 nM in NaCl)
- 0.100 ml Pefafluor FXa (1.5 mM in water)

=> Determination of fluorescence emission at 440 nm at 25°C

**Package size:** Vial containing 10 µmol

Bulk [g]

**Code:** 085-21

085-12

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.
**Pefafluor TH**

**Application:** Sensitive fluorogenic peptide substrate for thrombin. Determination of thrombin activity for research, in-process and quality control.

**Formula:** H-D-CHA-Ala-Arg-AMC-2AcOH  \[\text{MW: } 675.8\]

**K_M:** 1.93 \(\mu\)M  \(\text{Kcat: } 53.9 \text{ s}^{-1}\)

**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 micro-organisms. Shipment of product does not require cooling during the time of transportation.

**Assay:**

Suggested protocol for the determination of thrombin in microplates:

**Stock solutions:** Thrombin (3 NIH U/ml in 300 mM NaCl)

**Assay:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>bovine thrombin solution</td>
<td>0.070 ml</td>
</tr>
<tr>
<td>0.05 M Tris/HCl, 300 mM NaCl, 0.5% HSA, pH 8.4</td>
<td>0.830 ml</td>
</tr>
<tr>
<td>Pefafluor TH (2 mM)</td>
<td>0.100 ml</td>
</tr>
</tbody>
</table>

Determination of fluorescence emission at 460 nm

**Excitation wavelength 360 nm / Emission wavelength 460 nm**

**Package size:** Vial containing 25 mg

**Bulk [g]**

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.

**Pefafluor tPA**

**Application:** Highly sensitive fluorogenic peptide substrate for tissue-type plasminogen activator (tPA).

**Formula:** CH_3SO_2-3-Phe-Gly-Arg-AMC-AcOH  \[\text{MW: } 673\]

**K_cat:** 11.0 s^{-1}  \(\text{K_M: } 0.14 \text{ mM}\)

**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 micro-organisms. Shipment of product does not require cooling during the time of transportation.

**Assay:**

Suggested protocol for the determination of sc-tPA activity:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>sc-tPA (0.5 (\mu)M)</td>
<td>0.025 ml</td>
</tr>
<tr>
<td>Pefafluor tPA (1.0 mM in water)</td>
<td>0.100 ml</td>
</tr>
</tbody>
</table>

Determination of fluorescence emission at 440 nm at 25°C

**Package size:** Vial containing 25 mg

**Bulk [g]**

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.
**Pefafluor uPA**

**Application:** Highly sensitive fluorogenic peptide substrate for urokinase (uPA).

**Formula:** Bz-β-Ala-Gly-Arg-AMC-AcOH  
**MW:** 623.7

**Formula:**  
**v\text{max}:** 0.048 µmol · l⁻¹ · s⁻¹  
**K_M:** 0.050 mM

**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 micro-organisms. Shipment of product does not require cooling during the time of transportation.

**Assay:** Suggested protocol for the determination of urokinase:

0.200 ml 50 mM Tris-HCl pH 8.0, 100 mM NaCl  
0.025 ml uPA (Ribosepharm, 850 IU/mg)  
0.025 ml Pefafluor uPA (0.5 mM in water)

=> Determination of fluorescence emission at 440 nm at 25°C

**Package size:** Vial containing 10 µmol  
**Code:** 082-21  
Bulk [g] 082-03

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.

**Pefafluor PCa**

**Application:** Sensitive fluorogenic peptide substrate for activated protein C.

**Formula:** Pyr-Pro-Arg-AMC-AcOH  
**MW:** 599.6

**Formula:**  
**k_cat:** 62.0 s⁻¹  
**K_M:** 0.56 mM

**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 micro-organisms. Shipment of product does not require cooling during the time of transportation.

**Assay:** Suggested protocol for the determination of activated protein C:

0.850 ml 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.5% HSA  
0.050 ml activated protein C (KORDIA, 46 nM)  
0.100 ml Pefafluor PCa (10 mM in water)

=> Determination of fluorescence emission at 440 nm at 25°C

**Package size:** Vial containing 25 mg  
**Code:** 089-05  
Bulk [g]

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.
**Pefafluor PCa3342**

**Application:** Sensitive chromogenic peptide substrate with significantly improved selectivity for activated protein C.

**Formula:** Pyr-CHG-Arg-AMC-AcOH  \[ \text{MW: } 641.7 \]

**\( k_{\text{cat}} \):** 17.0 s\(^{-1}\)  \[ \text{K}_M: \text{1.60 mM} \]

**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. Shipment of product does not require cooling during the time of transportation.

**Assay:** Suggested protocol for the determination of activated protein C:

- 0.850 ml 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.5% HSA
- 0.050 ml Activated protein C (KORDIA, 46 nM)
- 0.100 ml Pefafluor PCa3342 (1.5 mM in water)

=> Determination of fluorescence emission at 440 nm at 25°C

**Package size:** Vial containing 25 mg

**Code:** 089-10

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.

**Pefafluor LAL**

**Application:** Highly sensitive fluorogenic peptide substrate for the determination of bacterial endotoxins.

**Formula:** CH\(_3\)SO\(_2\)-D-CHA-Gly-Arg-AMC-AcOH  \[ \text{MW: } 695.8 \]

**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. Shipment of product does not require cooling during the time of transportation.

**Package size:** Vial containing 25 mg

**Code:** 086-05

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.
Pefafluor FIXa10148

**Application:** Fluorogenic peptide substrate for factor IXa with improved sensitivity. Determination of factor IXa activity for in-process and quality control of factor IX preparations.

**Formula:** Mes-(D)-CHG-Gly-Arg-AMC AcOH  
**MW:** 665.7

**V<sub>max</sub>:** 28.1 µmol/min  
**K<sub>M</sub>:** 0.23 mM  
(determined in the presence of 33% ethylene glycol)

**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. Shipment of product does not require cooling during the time of transportation.

**Assay:**

Suggested protocol for an assay in microplates:

<table>
<thead>
<tr>
<th>Buffer: 50 mM Tris pH 7.4, 100 mM NaCl, 5 mM CaCl&lt;sub&gt;2&lt;/sub&gt;, 40% ethylene glycol</th>
<th>Pefafluor FIXa10148 (10 mM in water)</th>
<th>FIXa: human factor IXaβ, Enzyme Research, final conc. 19.4 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.200 ml Buffer</td>
<td>0.025 ml Pefafluor FIXa10148</td>
<td>0.020 ml FIXa: human factor IXaβ, Enzyme Research, final conc. 19.4 µg/ml</td>
</tr>
</tbody>
</table>

**Note:** The sensitivity of Pefafluor FIXa10148 is significantly increased in the presence of 33% ethylene glycol.

**Package size:** Bulk [g]  
**Code:** 095-03

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.

**Pefafluor FIXa3668**

**Application:** Highly sensitive fluorogenic peptide substrate for factor IXa.

**Formula:** H-(D)-Leu-PHG-Arg-AMC 2AcOH  
**MW:** 696.8

**V<sub>max</sub>:** 1.12 µmol/min  
**K<sub>M</sub>:** 0.028 mM

**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. Shipment of product does not require cooling during the time of transportation.

**Assay:**

Suggested protocol for the determination of factor IXa activity:

<table>
<thead>
<tr>
<th>Buffer (50 mM Tris-HCl pH 7.4, 100 mM NaCl, 5 mM CaCl&lt;sub&gt;2&lt;/sub&gt;, 0.5% HSA)</th>
<th>Pefafluor FIXa3668 (10 mM in water)</th>
<th>FIXa: human factor IXaβ, Enzyme Research, final conc. 19.4 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025 ml Pefafluor FIXa3668</td>
<td>0.200 ml Buffer</td>
<td>0.020 ml FIXa: human factor IXaβ, Enzyme Research, final conc. 19.4 µg/ml</td>
</tr>
</tbody>
</table>

**Package size:** Bulk [g]  
**Code:** 095-04

FOR RESEARCH USE ONLY. NOT FOR HUMAN USE OR DRUG USE.