PENTAPHARM

Pefachrome® FIXa

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

Formula: CH_3SO_2-(D)-CHG-Gly-Arg-pNA·AcOH

k_{cat}/K_M: 3.38 l/mmol·s (determined in the presence of 33% ethylene glycol)

K_m: 1.3 mM **k_{cat}:** 4.4 s^{-1}

Solubility: Up to 20 mM in water **MW:** 628.7

Intended use: Determination of Factor IXa in concentrates or other blood products according to D. Prasa and J. Stuerzebecher, Thromb. Res. 1998;92:99-102

Principle: Peptide-pNA + F IXa ——————>Peptide-COOH + pNA (yellow)

Instrument: Spectrophotometer or microtiter plate reader

Wavelength: 405 nm

Preparation: Dissolve content of vial in 1 ml distilled water (concentration: 10 mM)
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 micro-organisms.
Shipment of product does not require cooling during the time of transportation.

Not provided: Test buffer, reference material

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

Registered as
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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. Don‘ t work with chilled reagents directly from the refrigerator. For the kinetic version 37°C may be used, especially when a thermostated 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.

Microtiter plate reader	Spectrophotometer
0.200 ml buffer	0.800 ml buffer
0.025 ml Pefachrome® FIXa (10 mM)	0.100 ml Pefachrome® FIXa (10 mM)
0.020 ml Sample (factor IXaβ, 2 μM)	0.080 ml Sample (factor IXaβ, 2 μM)
⇒ Determination of optical density at 405 nm	⇒ Determination of optical density at 405 nm
0.025 ml Acetic acid (50 %) to stop the reaction after 5-10 minutes	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)

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 above.

Interference by turbidity or from coloured 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

References:

Stürzebecher J, Kopetzki E, Bode W, Hopfner KP
Dramatic enhancement of the catalytic activity of coagulation factor IXa by alcohols.
FEBS Lett 1997; 412:295-300

Prasa D, Stuerzebecher J
Determination of activated factor IX in factor IX concentrates with a chromogenic substrate. Throm Res; 92:99-102

Package size: Vial containing 10 μmol **Code:** 095-20

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