Product Information

Thrombin generation chromogenic substrate

Catalog Number T3068
Storage Temperature 2–8 °C

Synonym: β-Ala-Gly-Arg p-nitroanilide diacetate

Product Description
Molecular formula: C_{21}H_{34}N_{8}O_{9}
Molecular weight: 542.54

Thrombin generation chromogenic substrate is a chromogenic peptide substrate that is specifically cleaved by thrombin but at a slow rate. For the continuous determination of thrombin formation in plasma, such a selective thrombin substrate is required, one with moderate binding affinities (high $K_M$) and a low turnover rate ($k_{cat}$).

Thrombin reaction:

$$\beta$$-Ala-Gly-Arg p-nitroanilide $\rightarrow$ β-Ala-Gly-Arg + p-nitroanilide

Reaction is monitored by determining the release of p-nitroaniline by measuring the absorbance at 405 nm ($E_{\text{M}} = 9.65 \pm 0.35$).

$K_M$: 1.95 mM
$k_{cat}$: 1.91 s$^{-1}$

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Reconstitution with 9.2 ml of water results in a 5 mM reaction solution.

Storage/Stability
Store the product at 2–8 °C.

After reconstitution, solutions may be stored at 2–8 °C for 1 week or at −20 °C for one month.

Procedure
Determination of thrombin generation in human plasma:

1. Combine:

   600 µl of platelet-free human plasma
   75 µl of 50 mM Tris-HCl, pH 7.4, with 100 mM NaCl and 0.5% human serum albumin
   75 µl of Gly-Pro-Arg-Pro solution (36 mg/ml, Catalog Number G1895)
   90 µl of Thrombin generation chromogenic substrate reaction solution (5 mM)

2. Incubate at 37 °C.

3. Add 60 µl of Innovin® (supplied by Siemens) in 250 mM CaCl$_2$ or 60 µl of Dapttin® (available from Technoclone) in 250 mM CaCl$_2$

4. Monitor release of p-nitroaniline by measuring absorbance at 405 nm for 15 minutes

References

Innovin is a registered trademark of Siemens Healthcare Diagnostics.
Dapttin is a registered trademark of Technoclone.

DXP,RBG,MAM 09/08-1