

## Product Information

### Thrombin generation chromogenic substrate

Catalog Number **T3068**

Storage Temperature 2–8 °C

Synonym:  $\beta$ -Ala-Gly-Arg *p*-nitroanilide diacetate

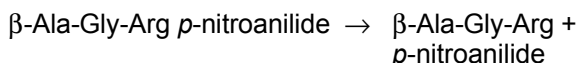
#### Product Description

Molecular formula: C<sub>21</sub>H<sub>34</sub>N<sub>8</sub>O<sub>9</sub>

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:



Reaction is monitored by determining the release of *p*-nitroaniline by measuring the absorbance at 405 nm ( $E^{mM} = 9.65 \pm 0.35$ ).

$K_M$ : 1.95 mM

$k_{cat}$ : 1.91 s<sup>-1</sup>

#### 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  $\mu$ l of platelet-free human plasma
  - 75  $\mu$ l of 50 mM Tris-HCl, pH 7.4, with 100 mM NaCl and 0.5% human serum albumin
  - 75  $\mu$ l of Gly-Pro-Arg-Pro solution (36 mg/ml, Catalog Number G1895)
  - 90  $\mu$ l of Thrombin generation chromogenic substrate reaction solution (5 mM)
2. Incubate at 37 °C.
3. Add 60  $\mu$ l of Innovin® (supplied by Siemens) in 250 mM CaCl<sub>2</sub> or 60  $\mu$ l of Daptin® (available from Technoclone) in 250 mM CaCl<sub>2</sub>
4. Monitor release of *p*-nitroaniline by measuring absorbance at 405 nm for 15 minutes

#### References

1. Prasa, D. et al., The ability of thrombin inhibitors to reduce the thrombin activity generated in plasma on extrinsic and intrinsic activation. *Thromb. and Haemost.*, **77**, 498-503 (1997).
2. Prasa, D. et al., Inhibition of Thrombin Generation in Plasma by Inhibitors of Factor Xa. *Thromb. and Haemost.*, **78**, 1215-1220 (1997).

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