



MP Biomedicals, LLC

29525 Fountain Parkway  
Solon, Ohio 44139

Telephone: 440/337-1200  
Toll Free: 800/854-0530  
Fax: 440/337-1180  
mailto: [biotech@mpbio.com](mailto:biotech@mpbio.com)  
web: <http://www.mpbio.com>

## TECHNICAL INFORMATION

### ENZYME SYSTEMS PRODUCTS

a division of



### Technical Data Sheet

#### Method for Assay of Thrombin or Kallikrein with D-Pro-Phe-Arg

##### Background:

Thrombin cleaves - Arg-Gly bonds in the a and b chain of fibrinogen, liberating N-terminal fibrinopeptides to generate fibrin. Also acts on factors V, VII, VIII and XIII, always cleaving arginyl bonds. Some low molecular weight substrates of trypsin are susceptible to thrombin. P<sub>1</sub> of human thrombin 7.0-7.6.

##### Material:

50 mM Tris, 100 mM NaCl, 10 mM CaCl<sub>2</sub>, pH 8

##### – Buffer

20 mM solution of D-Pro-Phe-Arg (Catalog # AFC- or AMC059) in DMSO

##### – Substrate

Cells lysate or purified enzyme solution (~40 NIH Units)

##### – Enzyme

80 μM free substrate (ESP catalog # T07 or T06) in DMSO

##### – Fluorescence Standard

##### Method:

1. Add 10 μl of enzyme to 490 μl of buffer. Mix. Incubate at 30° C for 15 minutes. If using an inhibitor, add inhibitor and incubate an additional 15 minutes.
2. With fluorometer, adjust to approximately 400 nm excitation, 505 emission. Add 20 μl of substrate enzyme solution.
3. Record increase in fluorescence from T<sub>0</sub> to T<sub>end</sub> where fluorescence units generated at T<sub>end</sub> are significantly different from those at T<sub>0</sub>.
4. Record fluorescence units generated by 10, 20 and 30 μl of free AFC or AMC in 490, 480, and 470 μl buffer solution, respectively.
5. Graph fluorescence units generated by enzyme to activity.

##### Storage:

Desiccate AFC-, or AMC059 in solid form at room temperature. Store DMSO/DMF solution at -20° C. Material is stable for at least one year, if stored as recommended.

##### References:

- A.J. Barrett; J.K. McDonald; Mammalian Proteases 1:85 *Academic Press* (1980)