

Data Sheet

Fluorogenic FAP Assay Kit

Catalog #: 80210

DESCRIPTION: Fibroblast Activation Protein, or FAP, is a 170 kDa dimer with dipeptidase activity. FAP expression is seen on activated stromal fibroblasts in >90% of all human carcinomas, suggesting a role in the development, growth and metastasis of carcinomas. It is also involved in the control of wound healing and tissue repair. The *Fluorogenic FAP Assay Kit* is designed to measure FAP activity using purified FAP for screening and profiling applications. It comes in a convenient 96-well format, with purified FAP enzyme, DPP substrate, and DPP assay buffer for 100 enzyme reactions. The key to the *Fluorogenic FAP Assay Kit* is the fluorogenic substrate. Using this kit, only one simple step on a microtiter plate is required for FAP reactions. The DPP fluorometric substrate is incubated with a sample containing FAP enzyme to produce a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80100	FAP human recombinant enzyme	30 µg	-80°C	Avoid freeze/thaw cycles!
80300	DPP assay buffer	10 ml	-20°C	
80305	Fluorogenic DPP substrate 1 in DMSO (0.5 mM)	100 µl	-80°C	
	AMC Fluorescent standard (50 µM)	500 µl	-20°C	
79685	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

REFERENCES:

1. Edosada, C.Y. et al., *J. Biol. Chem.* **281 (11)**: 7437-7444 (2006).
2. Edosada, C.Y. et al., *FEBS Lett.* **580 (6)**: 1581-1586 (2006).

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ASSAY PROTOCOL:**Immediately prior to assay:**

- 1) Dilute DPP substrate 0.5 mM stock 5-fold with DPP assay buffer to make a 100 μ M solution. (Make only sufficient quantity needed for the assay; store remaining 0.5 mM stock solution in aliquots at -20°C.)
- 2) Dilute FAP in DPP assay buffer to 25 ng/ μ l (250 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. **Note: Optimal enzyme concentration may vary with the specific activity of the enzyme.*
- 3) Dilute 25 μ l of the Fluorescent AMC standard (50 μ M stock) 2-fold with DPP buffer to make a 25 μ M solution. Make serial 2-fold dilutions of the fluorescent AMC standard in DPP buffer as follows: 12.5 μ M, 6.25 μ M, 3.12 μ M, 1.56 μ M, 0.78 μ M, 0.39 μ M, 0.19 μ M, 0.10 μ M. Aliquot the remaining 50 μ M AMC standard and store undiluted at -20°C.

Step 1:

In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at 22 °C for 10 min.

	Enzyme Positive Control	Test Inhibitor	AMC Standard Curve	Inhibitor Negative Control	“Blank” Negative Control
FAP (25 ng/ μ l)	10 μ l	10 μ l	–	–	–
DPP substrate (100 μ M)	5 μ l	5 μ l	–	5 μ l	–
AMC standard (0.1 μ M – 50 μ M)	–	–	5 μ l	–	–
Inhibitor (in DPP assay buffer)	–	X μ l	–	X μ l	–
DPP assay buffer	85 μ l	85 - X μ l	95 μ l	95 - X μ l	100 μ l
Total	100 μl	100 μl	100 μl	100 μl	100 μl

Step 2:

Read sample in a microtiter-plate fluorimeter that is capable of excitation at wavelengths ranging from 350-380 nm and detection of emitted light ranging from 440-460 nm. “Blank” value is subtracted from all other values.

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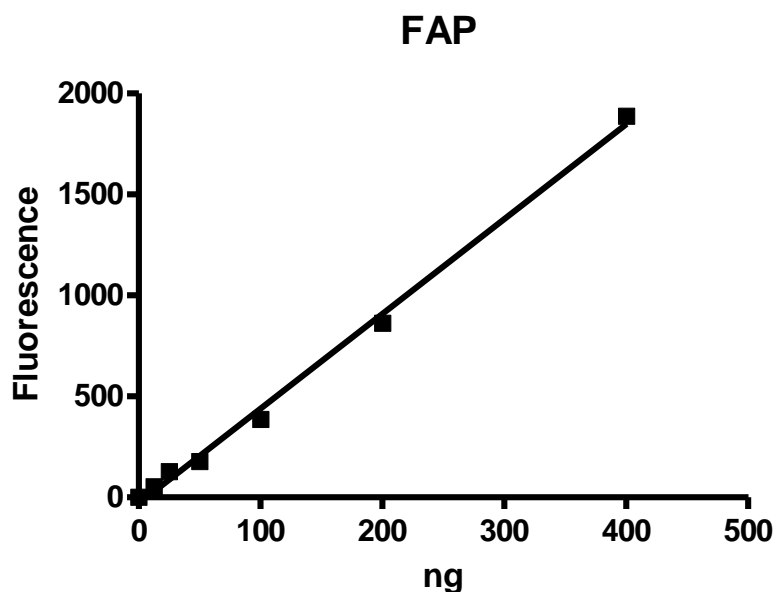
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Example of Assay Results:



FAP enzyme activity, measured using the *Fluorogenic FAP Assay Kit*, BPS Bioscience Cat.# 80100. *Note: Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
FAP enzyme	80100	10 µg
DPP assay buffer	80300	20 ml
Fluorogenic DPP substrate 1	80305	100 µl
DPP3 enzyme	80030	10 µg
DPP4 enzyme	80040	10 µg
DPP7 enzyme	80070	10 µg
DPP8 enzyme	80080	10 µg
DPP9 enzyme	80090	10 µg
POP enzyme	80105	20 µg
Fluorogenic DPP3 assay kit	80203	96 rxns
Fluorogenic DPP4 assay kit	80204	96 rxns
Fluorogenic DPP7 assay kit	80207	96 rxns
Fluorogenic DPP8 assay kit	80208	96 rxns
Fluorogenic DPP9 assay kit	80209	96 rxns
Fluorogenic POP assay kit	80106	96 rxns
Fluorogenic DPP substrate 2	80332	100 µl

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