Description

The FAP Fluorogenic Assay Kit is designed to measure FAP (fibroblast activation protein) protease activity for screening and profiling applications. It comes in a convenient 96-well format, with purified recombinant FAP enzyme (amino acids 29-760), DPP substrate, and DPP assay buffer for 100 enzyme reactions.

The DPP fluorogenic substrate is incubated with a sample containing FAP enzyme to produce a fluorophore that can then be measured using a fluorescence reader.

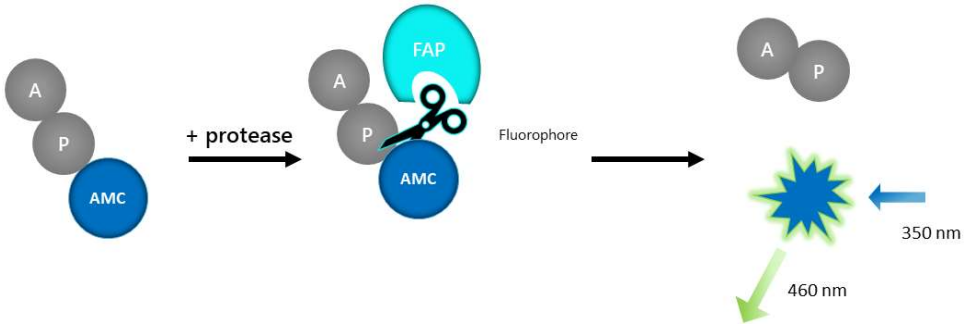

Figure 1: Illustration of the assay principle.

Fluorogenic DPP Substrate 1 is a fluorogenic peptide substrate (Ala-Pro-AMC dipeptide). In the conjugated form the energy emitted from the fluorochrome AMC is quenched. Proteolysis releases AMC and fluorescence is emitted. The increase in fluorescence is directly proportional to FAP activity.

Background

Fibroblast Activation Protein, or FAP, is a type II membrane serine protease of the dipeptidyl peptidase (DPP) subfamily. It is involved in wound healing and tissue repair. FAP upregulation is seen on activated stromal fibroblasts present in cancer, including breast, lung, prostate, pancreatic and cervical cancer. It has an impact on the tumor microenvironment (TME), where it reduces the levels of PEDF (pigment epithelium derived factor), angiopoietin-1 and VEGF-c (vascular endothelial growth factor c) and increase TGF-β (transforming growth factor β) and create an immunosuppressive environment. It is used as biomarker for pro-tumorigenic stroma and links to a poor prognosis. Studies in animal models have shown that knock-out of FAP reduces the metastasis of pancreatic ductal adenocarcinomas (PDA). It has thus been proposed as a candidate target for small molecule inhibitors for cancer therapy applications. Additionally, its expression profile makes it a good candidate for CAR-T applications, with pre-clinical and clinical trials currently ongoing.

Applications

Enzyme kinetics studies and screening small molecule inhibitors for drug discovery and high-throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
80100	FAP, His-Tag*	30 µg	-80°C
80300	DPP Assay Buffer	10 ml	-20°C
80305	0.5 mM Fluorogenic DPP Substrate 1	100 µl	-80°C
	50 µM AMC Fluorescent Standard	500 µl	-20°C
79685	96-well black microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

Fluorescent microtiter plate reader capable of measurement at λex350-380/λem440-460 nm

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Negative Control", "Positive Control", "AMC Standard Curve" (ranging from 12.5 µM to 0.10 µM) and "Test Inhibitor" conditions.
- If the assay plate is going to be used more than once, prepare enough of each reagent for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or as recommended for each reagent.
- ***Unused diluted proteins should be discarded.***

1. Thaw **FAP** on ice. Briefly spin the tube to recover its full content.

2. Dilute FAP to 25 ng/µl with DPP Assay Buffer (10 µl/well). Aliquot any remaining enzyme and store undiluted at -80°C.

   Note: Keep the diluted protein on ice until use. Do not freeze and re-use the diluted protein.

3. Add 10 µl of diluted enzyme to the "Positive Control" and "Test Inhibitor" wells.

4. Dilute 25 µl of the **50 µM Fluorescent AMC Standard** 2-fold with DPP Assay Buffer to make a 25 µM solution.

5. Prepare serial 2-fold dilutions of the diluted Fluorescent AMC Standard in DPP Assay Buffer, ranging from 12.5 µM to 0.10 µM.

6. Add 5 µl of each dilution to the "AMC Standard Curve" wells.

7. Add 95 µl of DPP Assay Buffer to the "AMC Standard Curve" wells.

8. Prepare the Test inhibitor (10 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 100 µl.

   9.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in DPP Assay Buffer at concentrations 10-fold higher than the desired final concentrations.

   OR

   9.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in DPP Assay Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using DPP Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in DPP Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO in the assay should not exceed 1%.

9. Add 10 µl of Test inhibitor to each well designated "Test Inhibitor" and "Negative Control".

10. Add 10 µl of Diluent Solution to the "Positive Control" and "Blank" wells.

11. Add 75 µl of DPP Assay Buffer to the "Test Inhibitor" and "Positive Control" wells.

12. Add 85 µl of DPP Assay Buffer to the "Negative Control".

13. Add 90 µl of DPP Assay Buffer to the "Blank" wells.

14. Dilute 5-fold the **0.5 mM Fluorogenic DPP Substrate 1** with **DPP Assay Buffer** to make a 100 µM solution.

15. Initiate reaction, by adding 5 µl of diluted Fluorogenic DPP Substrate 1 to the "Positive Control", "Test Inhibitor" and "Negative Control" wells.

16. Incubate at room temperature for 10 minutes.

17. Read the fluorescence intensity of the samples (excitation = 350-380 nm; emission = 440-460 nm) in a fluorescence reader.

18. The "Blank" value should be subtracted from all other values.

Component	Blank	Negative control	Positive Control	Test Inhibitor	AMC Standard Curve
Diluted FAP (25 ng/µl)	-	-	10 µl	10 µl	-
Diluted Fluorescent AMC Standard	-	-	-	-	5 µl
Test Inhibitor	-	10 µl	-	10 µl	-
Diluent Solution	10 µl	-	10 µl	-	-
DPP Assay Buffer	90 µl	85 µl	75 µl	75 µl	95 µl
Diluted Fluorogenic DPP Substrate 1 (100 µM)	-	5 µl	5 µl	5 µl	-
Total	**100 µl**	**100 µl**	**100 µl**	**100 µl**	**100 µl**

Example Results

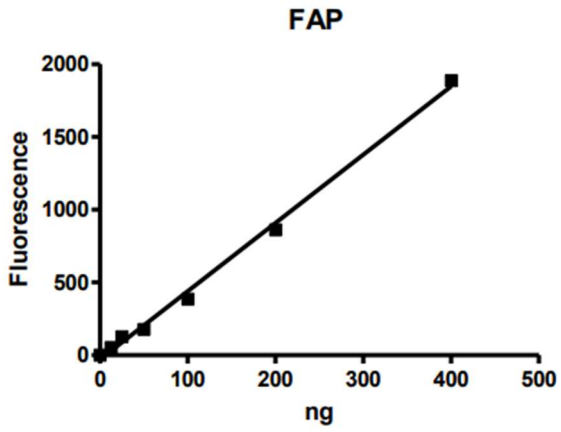

Figure 2. FAP activity.
FAP activity was measured in the presence of increasing concentrations of FAP.

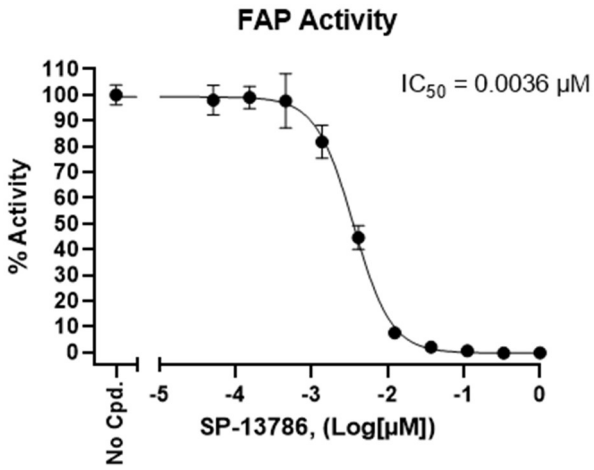

Figure 3. Inhibition of FAP activity by SP-13786.
FAP activity was measured in the presence of increasing concentrations of SP-13786 (Selleckchem #S0842). Results are expressed as percent activity, in which the activity of FAP in the absence of inhibitor is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References
Pure E. and Blomberg R., 2018, *Oncogene* 37: 4343-4357.
Bughda R., *et al.*, 2021, *Immunotargets Ther.* 10:313-323.

Related Products

Products	*Catalog #*	*Size*
FAP- CHO K1 Recombinant Cell Line	79947	2 vials
FAP, His-Tag (Mouse) Recombinant	100226	25 µg