SensoLyte® AMC DPPIV Assay Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72197**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect DPPIV activity.
- ***Enhanced Value:*** Ample reagents to perform 100 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in one hour
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	DPPIV Substrate, Ex/Em=354/442 nm upon substrate cleavage	55 µL
Component B	AMC, fluorescence reference standard, Ex/Em=354 /442 nm	5 mM, 10 µL
Component C	DPPIV, porcine kidney	0.1 mg/mL, 15 µL
Component D	Assay Buffer	25 mL
Component E	DPPIV Inhibitor	10mM, 15 µL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat bottom 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 442 nm with excitation at 354 nm.

Storage and Handling

- Store all kit components at -20°C, except for Component C
- Store Component C at -80°C
- Protect Components A and B from light and moisture
- Component D can be stored at room temperature for convenience

Introduction

Dipeptidyl Peptidase IV (DPPIV, DPP-4, CD26) is cell a surface serine protease, which cleaves Xaa-Pro or less efficiently Xaa-Ala dipeptides from the N-terminus.[1-3] DPPIV possess an identical function to the T-cell surface antigen (CD26).[1] DPPIV is commonly found in mammalian tissues and is highly expressed in hepatocytes, pancreatic epithelial cells, intestinal epithelium, and kidney cortex.[3] DPPIV plays an important role in glucose homeostasis by proteolysis of incretins such as glucagon-like peptide-1 (GLP-1). Due to its role in degradation of GLP-1, DPPIV is a therapeutic target for type 2 diabetes.

The SensoLyte® AMC DPPIV Assay Kit is optimized for screening of enzyme inhibitors. This kit contains a fluorogenic substrate that has a high reactivity and low background. Upon protease cleavage, this substrate generates the AMC fluorophore emitting bright blue fluorescence that can be monitored at excitation/emission=354/442 nm. Increase in AMC fluorescence is proportional to DPPIV activity.

Protocol

Screening DPPIV inhibitors using purified enzyme

Note: For standard curve, please refer to Appendix II (optional).

1. Prepare working solutions.

Note: Bring all kit components until thawed to room temperature before starting the experiments.

1.1 DPPIV substrate solution: Dilute DPPIV substrate (Component A) 100-fold in assay buffer. Prepare fresh assay buffer for each experiment. Refer to Table 1.

Table 1. DPPIV substrate solution for one 96-well plate (100 assays).

Components	Volume
DPPIV substrate (100X, Component A)	50 µL
Assay buffer	4.95 mL
Total volume	5 mL

1.2 DPPIV diluent: Dilute the enzyme (Component C) 1:400 in assay buffer. This amount of enzyme is enough for a full 96-well plate. If not using the entire plate, adjust the amount of enzyme to be diluted accordingly. Store the remainder of the undiluted enzyme at –80C.

Note: Prepare enzyme diluents immediately before use. Do not vortex the enzyme solutions. Prolonged storage or vigorous agitation of the diluted enzyme will cause denaturation. Store the diluted enzyme solution on ice.

1.3 DPPIV inhibitor (P32/98 – competitive, substrate analog inhibitor of DPPIV): Dilute the 10 mM inhibitor solution (Component E) 1:100 in assay buffer. The diluted inhibitor solution has a concentration of 100 µM. Add 10 µl of the solution into each of the inhibitor control well.

2. Set up the enzymatic reaction.

2.1 Add test compounds and diluted enzyme solution to the microplate wells. The suggested volume of enzyme solution for a 96-well plate is 40 µL/well and test compound is 10 µL/well.

2.2 Simultaneously set up the following control wells, as deemed necessary:

- Positive control contains the enzyme without test compound.
- Inhibitor control contains DPPIV enzyme and inhibitor.
- Vehicle control contains DPPIV enzyme and vehicle used in delivering test compound (e.g. DMSO, concentration not to exceed 1%).

- Test compound control contains assay buffer and test compound. Some test compounds have strong autofluorescence and may give false results.
- Substrate control contains assay buffer.

2.3 Using the assay buffer, bring the total volume of all controls to 50 µL.

2.4 Optional: Pre-incubate the plate for 10 min. at assay temperature. Any temperature (the *assay temperature*) from room temperature to 37°C may be used, as long as the subsequent incubations are performed at the same temperature.

3. Run the enzymatic reaction.

3.1 Add 50 µL of DPPIV substrate solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=354 nm/442 nm continuously and record data every 5 min. for 30 to 60 min.
- For end-point reading: Incubate the reaction for 30 to 60 min. Keep plate from direct light. Then measure fluorescence intensity at Ex/Em=354/442 nm.

3.3 For methods of data analysis: Refer to Appendix I.

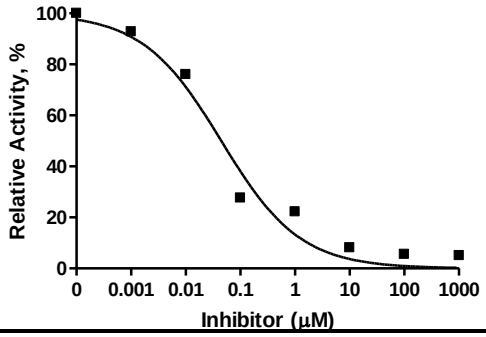

Figure 1. Inhibition of DPPIV activity measured with SensoLyte® AMC DPPIV Assay Kit.

Appendix I. Data Analysis

- The fluorescence reading from the substrate control well is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells containing substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).

- For kinetics analysis:
  - Plot data as RFU versus time for each sample.
  - Determine the range of initial time points during which the reaction is linear. Typically, the first 10-15% of the reaction will be the optimal range.
  - Obtain the initial reaction velocity (V_o) in RFU/min by determining the slope of the linear portion of the data plot.

- A variety of data analyses can be done, e.g., determining inhibition %, $EC_{50,}$ IC_{50}, K_m, K_i, etc.

- For endpoint analysis:
  - Plot data as RFU versus concentration of test compounds.
  - A variety of data analyses can be done, e.g., determining inhibition %, EC50, IC50, etc.

Appendix II. Instrument Calibration

- AMC fluorescence reference standard: Dilute 5 mM AMC (Component B) to 50 μM (1:100) in assay buffer. Do 2-fold serial dilutions to get concentrations of 25, 12.5, 6.25, 3.13, 1.57 and 0.79, include an assay buffer blank. Add 50 μL/well of these serially diluted AMC reference solutions.
- Add 50 μL/well of the diluted DPPIV substrate solution (refer to step 1.1 for preparation).

  Note: DPPIV substrate solution is added to the AMC reference standard to correct for the absorbance reading from the fluorogenic substrate. If multiple concentrations of substrate are used, this step must be performed for each concentration.
- Measure the fluorescence intensity of the reference standard wells at Ex/Em=354 nm/442 nm. Use the same sensitivity setting as used in the enzyme reaction.
- Plot the AMC fluorescent reference standard curve as RFU (relative fluorescent units) versus concentration as shown in Figure 2.
- The final concentrations of AMC reference standard are 25, 12.5, 6.25, 3.13, 1.57, 0.79, 0.39, and 0 μM. This reference standard is used to calibrate the variation of different instruments and different experiments. It is also an indicator of the amount of final product of the enzymatic reaction.

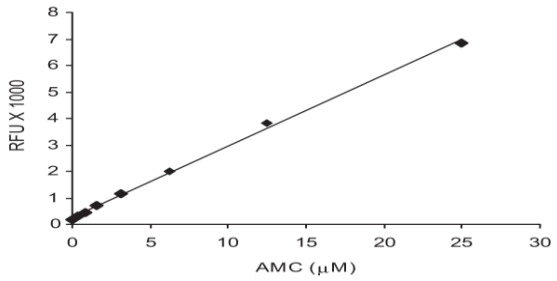

Figure 2. AMC reference standard. AMC was serially diluted in assay buffer containing substrate, and the fluorescence was recorded at Ex/Em=354/442 nm. (FLx800, Bio-Tek

References

1. Zhu, L. et al, *J. Biol. Chem.* 278, 22418 (2003).
2. Mentlein, R. *Regulatory Peptides* 85, 9 (1998).
3. Qi, SY. et al, *Biochem. J.* 373, 179 (2003).