



SensoLyte[®] Rh110 DPPIV Assay Kit *Fluorimetric*

Revision Number: 1.1	Last updated: October 2014
Catalog #	AS-72196
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=490/520 nm upon cleavage	55 μ L
Component B	Rh110, fluorescence reference standard, Ex/Em=490 nm/520 nm	5 mM, 10 μ L
Component C	DPPIV, porcine kidney	0.1 mg/mL, 15 μ L
Component D	Assay Buffer	25 mL
Component E	DPPIV Inhibitor	10mM, 15 μ L

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 520 nm with excitation at 490 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 a cell surface serine protease, which cleaves Xaa-Pro or less efficiently Xaa-Ala dipeptides from the N-terminus.¹⁻³ DPPIV possesses 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[®] Rh110 DPPIV Activity Assay Kit provides a convenient assay for screening of enzyme inhibitors and activators or for continuous assay of enzyme activity using a fluorogenic substrate. Upon cleavage by DPPIV, this substrate generates the Rh110 (rhodamine 110) fluorophore which has a bright green fluorescence and can be detected at excitation/emission=490/520 nm. The longer wavelength spectra and higher extinction coefficient of Rh110 provide greater sensitivity and less interference from other reaction components.

Protocol

Screening DPPIV inhibitors using purified enzyme

Note: For instrument calibration, 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 μ L
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 -80°C .

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 μ M. Add 10 μ l 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 μ L/well and test compound is 10 μ L/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=490 nm/520 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=490/520 nm.

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

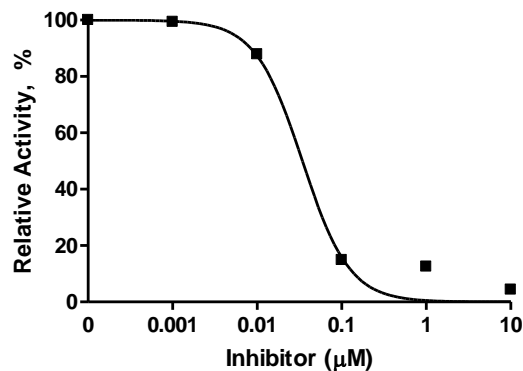


Figure 1. Inhibition of DPPIV activity measured with SensoLyte[®] Rh110 DPPIV Assay Kit.

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 %, EC_{50} , IC_{50} , etc.

Appendix II. Instrument Calibration

- Rh110 fluorescence reference standard: Dilute 5 mM Rh110 (Component B) to 50 μ M in assay buffer. Do 2-fold serial dilutions to get concentrations of 25, 12.5, 6.25, 3.13, 1.57, and 0.79 μ M, include an assay buffer blank. Add 50 μ L/well of these serially diluted Rh110 reference solutions.
- Add 50 μ L/well of the diluted DPPIV substrate solution (refer to step 1.1 for preparation).

Note: DPPIV substrate solution is added to the reference standard for normalizing the intrinsic substrate fluorescence. If multiple concentrations of substrate are used, this step must be performed for each concentration.
- Measure the fluorescence of the reference standard and substrate control wells at $Ex/Em=490/520$ nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot the Rh110 reference standard curve as RFU (relative fluorescent units) versus concentration.
- The final concentrations of Rh110 reference standard are 25, 12.5, 6.25, 3.13, 1.57, 0.79, 0.39, and 0 μ M. This reference standard is used to calibrate the variation of different instruments and different experiments. Since the proteolytic cleavage of the Rh110 substrate consists of two steps, with both the intermediate and final products having fluorescence, the Rh110 reference standard cannot serve as an indicator of the amount of enzymatic reaction final product.

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).