ab284508 – DPP4 Inhibitor Screening Kit (Fluorometric)

For the screening of potential DPP4 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284508

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
DPP4 Assay Buffer	25 mL	-20°C
DPP4 Substrate	200 µL	-20°C
DPP4 Enzyme	100 µL	-20°C
DPP4 Inhibitor (Sitagliptin)	50 µL	-20°C

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

DPP4 Assay Buffer: Warm to room temperature (RT) before use. Store at 4°C or -20°C.

DPP4 Inhibitor: Store at -20°C. Use within 2 months.

DPP4 Enzyme & DPP4 Substrate: Upon thawing aliquot and store at -20°C, use within 2 months.

Assay Protocol

Enzyme Solution preparation:

1. For each well, prepare a 50 µL DPP4 Enzyme Solution:

	Enzyme Solution
DPP4 Assay Buffer	49 µL
DPP4 Enzyme	1 µL

Screen test inhibitors, inhibitor control and blank control Preparations:

1. Dissolve test inhibitors into proper solvent.
2. Dilute to 4X the desired test concentration with DPP4 Assay Buffer.
3. For DPP4 Inhibitor Control, dilute1:9 with DPP4 Assay Buffer

Δ Note: *to compare test inhibitors to Inhibitor Control at its IC_{50}, dilute Inhibitor Control 1:99, then use 25 µL).*

4. Add 25 µL of test inhibitors, DPP4 Inhibitor or DPP4 Assay Buffer into DPP4 enzyme wells as sample screen (S), Inhibitor Control (IC), or Enzyme Control (EC).
5. Mix well, and incubate for 10 minutes at 37°C.

Substrate Mix:

1. For each well, prepare a total 25 µL Substrate Solution:

	Substrate Solution
DPP4 Assay Buffer	23 µL
DPP4 Substrate	2 µL

2. Mix well and incubate at 37°C

Measurement

Measure the fluorescence (Ex/Em = 360/460 nm) in kinetic mode for 15 - 30 minutes at 37°C. Protect from light. Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

Calculation

1. Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (= RFU_2 - RFU_1) values by the time ΔT (= T_2-T_1).
2. Calculate % Relative Inhibition as follows:

$$\% \text{ Relative inhibition} = \frac{(slope\ of\ EC - slope\ of\ S)}{slope\ of\ EC} \times 100$$

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)