SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

PAD1 Inhibitor Screening Kit

Catalog # P310G-863

Product Description

The PAD1 Inhibitor Screening Kit is a direct Enzyme-Linked ImmunoSorbent Assay (ELISA) kit for use in PAD1-targeted inhibitor profiling assays. The kit includes recombinant full-length human PAD1 expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag (accession number NM_013358). The kit also includes the following components:

Components – Dry Ice

SignalChem Cat#	Component Name	Size
P312-310G	PAD1, Active	50µg
P312-37C	PAD Cocktail, Active (0.5ug/µL)	5µg
P312-58	PAD Substrate, lyophilized	0.5mg
T575-31N	Trypsin, Active (10mg/ml)	200µg
D86-09	DTT Solution (1M)	10µl

Storage and Stability

Store product at –20°C or –70°C as specified on individual component labels. For PAD enzymes, avoid repeated handling and multiple freeze/thaw cycles.

Components – Kit Box

SignalChem Cat#	Component Name	Size
AB99-61DM	Detection Antibody, 500X	25ul
CP01-506	NeutrAvidin™ Coated Plate	1 plate
CS01-506	TMB Substrate	6ml
P312-09	PAD Buffer	8ml
SS01-09	Stop Solution	3ml
T01-09	Trypsin Digestion Buffer	15ml
WB20-09	Wash Buffer, 20X	2 x 30ml

Storage and Stability

Store kit box and contents at 4°C.

Other Materials Required

- Test compounds
- Milli-Q water or other source of pure water
- Pipettes or multichannel pipettes
- Plate cover and sealer
- Incubator for 37°C incubation
- Microplate reader capable of reading absorbance at 450nm and 540nm

Short Protocol

The following is only a short protocol. For a more detailed protocol, see the PAD1 Inhibitor Screening Kit Instruction Manual.

PAD-Trypsin ELISA Assay Protocol:

- Prepare coating solution by diluting PAD Substrate in 1X wash buffer to 0.5 ug/ml. For standard curve, perform 8-pt serial dilution in wash buffer.
- Place the NeutrAvidin™ coated strips onto ELISA plate frame. Wash 3X with 1X wash buffer. Invert plate and blot dry.
- Add 100uL coating solution per well. Incubate 1hr at RT.
- Decant solution from plate. Wash 4X with 1X wash buffer. Invert plate and blot dry after last wash.
- Dilute PAD1 enzyme in PAD buffer. Pre-incubate PAD1 enzyme with test compound(s) for 15-30min.
- Add 50uL enzyme solution (or buffer for standard curve and blank control), seal plate and incubate at 37°C for 20-60min.
- Repeat decanting/washing step.
- Add 100uL trypsin buffer per well and aspirate.
- Prepare trypsin digestion solution by diluting the stock 1000-fold in trypsin buffer. Add 100uL digestion solution into all wells except the standard curve (use buffer only).
- Repeat decanting/washing step.
- Dilute the Detection antibody 500X in wash buffer.
- Add 100uL antibody per well. Incubate 1hr at RT.
- Repeat decanting/washing step.
- Warm up the TMB substrate to RT prior to use.
- Add 50uL per well and incubate 20min at RT protected from light
- Add 50uL stop solution to each well. The color in the wells should change from blue to yellow. Tap the plate to ensure thorough mixing.
- Read OD at 450nm and 540nm in a microplate reader within 30min of reaction termination.

Applications Note

For specific PAD1 Inhibitor Screening Kit applications, visit the PAD1 Inhibitor Screening Kit page on the SignalChem website