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 (0.5µg/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	25µl
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 15ml

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 µg/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 100µL 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 50µL 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 100µL trypsin buffer per well and aspirate.
- Prepare trypsin digestion solution by diluting the stock 1000-fold in trypsin buffer. Add 100µL 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 100µL antibody per well. Incubate 1hr at RT.
- Repeat decanting/washing step.
- Warm up the TMB substrate to RT prior to use.
- Add 50µL per well and incubate 20min at RT protected from light
- Add 25µL 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

To place your order, please contact us by phone 1-866-9KINASE (54-6273), 1-(604)-232-4600,
fax 1-604-232-4601 or by email: orders@signalchem.com - www.signalchem.com