SensoLyte® Green Pin1 Activity Assay Kit
Fluorimetric

Revision number: 1.2	Last updated: 02/28/17
Catalog #	**AS-72240**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** This kit is optimized to detect Pin-1 activity.
- ***Enhanced Value:*** It provides enough reagents to perform 100 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed in 1 hour.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Pin1 Green Substrate	0.1 mM , 60 µL
Component B	Pin1, Human Recombinant	1 mg/ml, 10 µL
Component C	Pin1 Developer, 100X	50 µL, 4 vials
Component D	Assay Buffer	30 mL
Component E	Pin1 Inhibitor	50mM in DMSO, 25 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 520 nm with excitation at 490 nm.
- Plate cover: To prevent liquid evaporation during incubation step.

Storage and Handling

- Store kit Components A, C, D, and E at -20 °C. Store Component B at -80 °C.
- Protect Component A from light and from moisture.

Introduction

Peptidyl-prolyl isomerase (PPIase) Pin1 catalyzes *cis/trans* isomerization of the phospho-Serine/Threonine-Proline peptide bonds.[1-5] Pin1 consists of a short N-terminal protein-protein interaction domain that allows the enzyme to bind phosphoproteins and the longer C-terminal isomerase domain.[1-5] Pin1 has many biological substrates, plays a critical role in cell-cycle regulation, and is up-regulated in many human cancers.[1-5] Recently, Pin1 was linked to Alzheimer's disease pathogenesis.[1,2,4] Pin1 can bind phosphorylated Thr-212/231 residues of Tau protein and increase its deposphorylation that may prevent formation of Tau neurofibrilary tangles. [1,2,4]

The SensoLyte® Green Pin1 Assay Kit uses a fluorogenic substrate, pretreated to convert it into the *cis* isoform. Pin1 changes this substrate into the *trans* conformation that is readily cleaved to generate fluorescent signal. Fluorescence is then monitored at Ex/Em=490/520nm. Increase in fluorescence intensity is directly proportional to the Pin1 activity.

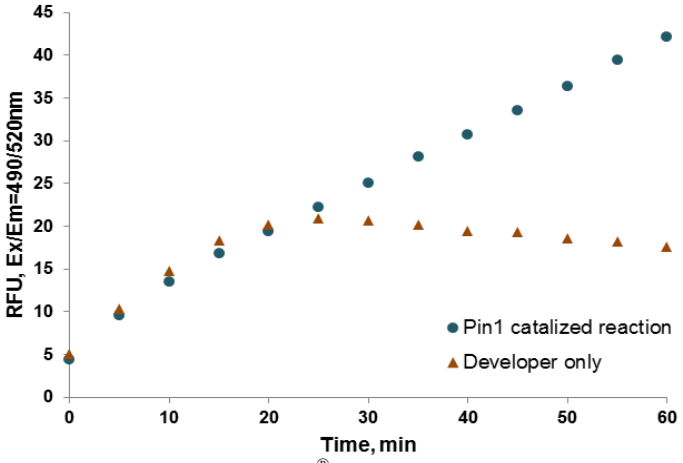

Figure 1. SensoLyte® Green Pin1 Activity example.

Protocol

1. Prepare working solutions.

Note 1: Allow all kit components to thaw before starting the experiment. Components B and C should be kept on ice.
Note 2: Briefly centrifuge Component B to completely recover enzyme.
Note 3: The amount of Pin1 enzyme is enough for 20 assays, the amounts supplied of the rest of the components are sufficient to run 100 assays. More Pin1 enzyme can be purchased at Abcam (Cat.#51230).
Note 4: Do not re-freeze and re-use Pin1 Developer (Component C).
Note 5: Suggested plate to run the assay: low binding, black opaque.

1.1 Pin1 substrate solution: Dilute Pin1 substrate (Component A) 1:100 with the assay buffer (Component D). This will provide a working substrate solution of 1 µM. 50 µL of this diluted substrate is enough for one-well reaction (96-well plate). For each experiment, prepare fresh substrate solution.

Table 1. Pin1 Substrate solution for one 96-well plate (100 assays).

Components	Volume
Pin1 substrate, 0.1 mM (Component A)	50 µL
Assay buffer (Component D)	4.95 mL
Total volume	5 mL

1.2 Recombinant Pin1 diluent: Dilute human enzyme (Component B) with 190 µl of the assay buffer (Component D) to a final concentration of 50 µg/ml. Suggested volume of the diluted enzyme solution for one well in a 96-well plate is 10 µL (500ng/well).

Table 2. Pin1 diluent for one 96-well plate (100 assays).

Components	Volume
Pin1 (Component B)	10 µL
Assay buffer (Component D)	190 µL
Total volume	200 µL

Note 1: Amount of recombinant enzyme (Component C) provided in this kit is enough for 20 assays at 500 ng/well of enzyme.
Note 2: Dilute enzyme immediately before use. Store diluted enzyme on ice.

1.3 Pin1 Inhibitor: Dilute Pin1 Inhibitor (Component E) 1:50 with the assay buffer (Component D) to a final concentration of 1mM. Suggested volume of the diluted inhibitor for one well in a 96-well plate is 10µl, final concentration 100µM/well.

1.4 Reaction Developer: Dilute Pin1 Developer (Component C) 1:100 with the assay buffer (Component D). Each assay well requires 30 µL of the prepared enzyme developer.

Table 3. Pin1 Developer solution for one 96-well plate (100 assays).

Components	Volume
Pin1 Developer, (Component C	30 µL
Assay buffer, (Component D)	2.97 mL
Total volume	3 mL

Note1: Prepare developer right before use. Otherwise keep prepared solution on ice until use. Do not re-freeze and re-use developer once thawed; entire vial should be used once de-frosted.

2. Set up the enzymatic reaction.

2.1 Add test compounds and enzyme solution to the microplate wells. For one well of a 96-well plate, the suggested volume of Pin1 enzyme solution is 10 µL and 10 µL of test compound.

2.2 Establish the following control wells at the same time, as deemed necessary:

- Positive control contains Pin1 enzyme without test compound.
- Inhibitor control contains Pin1 and Tannic acid.
- Vehicle control contains enzyme and vehicle used in delivering test compound (e.g. DMSO, concentration not to exceed 1%).
- Test compound control contains assay buffer (Component D) and test compound. Some test compounds may inhibit developer and be fluorescent or interfere with the Ex/Em wavelengths and thereby give false results.
- Background developer control contains assay buffer (Component D) without enzyme.

2.3 Using assay buffer (Component D), bring the total volume of all controls to 20 µL.

2.4 Add Pin1 Developer solution (from 1.4) at 30µl/well.

3. Run the enzymatic reaction.

3.1 Add 50 µL of Pin1 substrate (from 1.1) solution into each well. For best accuracy, it is advisable to have the substrate solution equilibrated to the assay temperature. Mix reagents completely by shaking the plate gently for no more than 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 60 to 120 min.

For end-point reading: Incubate the reaction at room temperature for 60 to 120 min. Keep plate away from direct light, then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

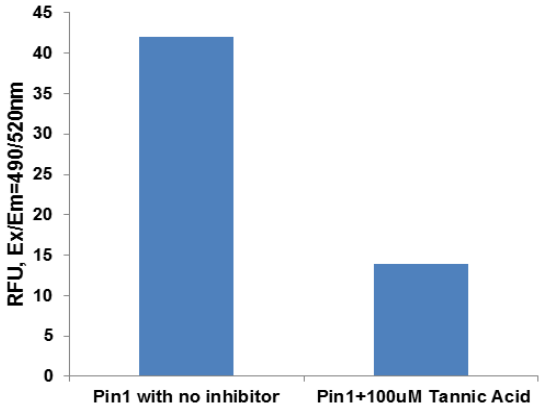

Figure 2. Tannic acid inhibition of Pin1 activity measured with SensoLyte® Green Pin1 Activity Assay Kit.

4. Data Analysis

4.1 Substrate will change its conformation into the more stable *trans* form on its own at slower rate. Therefore, readings from wells containing background developer control must be subtracted from other controls and test compound reading. All fluorescence readings are expressed in relative fluorescence units (RFU).

References

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5. Urusova D.V., et.al., Cancer Prev Res **4**, 9 (2011): 1366-1377