Signosis
Innovative Plate Assay Solutions

SH2 Domain-Based RTK Profiling Plate Array

Catalog Number DA-0001 **(For Research Use Only)**

Introduction

Receptor tyrosine kinases (RTKs) play important roles in converting extracellular to intracellular signals. Total 58 RTKs are identified in the human genome. They are activated by the binding of specific growth factors, cytokines, or hormones, which results in autophosphorylation. Tyrosine autophosphorylation generates recruitment sites for downstream signaling proteins containing Src homology-2 (SH2). Binding of different SH2 domain-containing signaling proteins represents the activation of different signaling pathways. Therefore, the activation of signaling pathways can be assessed through examination of 46 SH2 domain binding simultaneously. Comparison of SH2 domain binding of two samples could facilitate the discovery of difference in signaling pathways.

Principle of the assay

SH2 domain-based RTK profiling kit is an ELISA-like assay. SH2 domains are utilized for immobilization on the microtiter wells and an anti-phosphotyrosine antibody along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. Total 46 different SH2 domains are coated in different wells and 46 bindings can be measured. With a 96 well microplate, two samples can be compared among the bindings. The test sample is allowed to react simultaneously with the domain and the antibody. After incubation, the wells are washed to remove unbound-labeled antibody. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. Absorbance is measured spectrophotometrically at 450 nm.

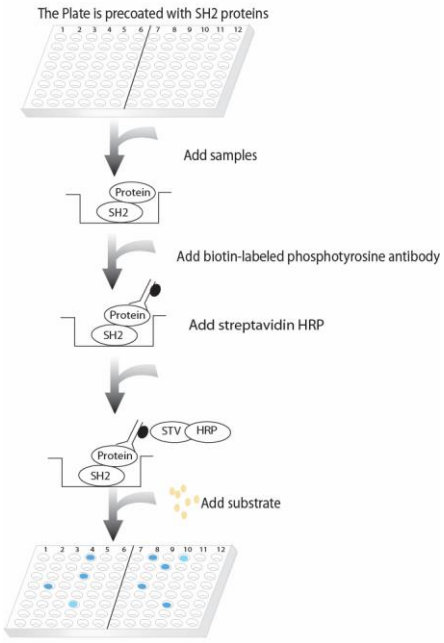

Diagram of SH2 domain-based RTK profiling

Materials provided with the kit

- 96-well microplate coated with 46 SH2 proteins (4ºC).
- 2X RTK Cell lysis buffer (-20ºC)
- 1X Diluent buffer (4ºC)
- Biotin phosphotyrosine antibody (-20ºC)
- Streptavidin-HRP conjugate (4ºC).
- 5X Assay wash buffer (4ºC)
- Substrate (4ºC).
- Stop Solution (4ºC).

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x Assay buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Use 2X RTK Cell Lysis buffer for tissue, or dilute to 1X RTK Cell Lysis buffer with ddH2O for cells
- Dilute 500 times of biotin Phosphotyrosine antibody with 1X Diluent buffer
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Sample preparation

1. One day prior to the experiment, the 2-5x10^6 cells are plated with the complete medium on 10cm^2 dish to reach 80-90% confluency on next day.
2. Before treatment, the cells are starved for 6 hr to 24 hours in 0.1% serum medium.
3. The cells are treated with appropriate stimuli for a given length of time.

Assay procedure

1. Sample Preparation

   Adherent Cells:

   Wash the cells with PBS and aspirate the PBS completely. Add 500ul of 1X RTK Cell Lysis buffer per 10cm^2 dish and incubate the plate on ice for 5 minutes. Scrape the cells and collect to a 1.5ml tube and sonicate briefly on ice. Centrifuge the tube for 2 minutes at 10,000 rpm. Transfer the supernatant to a fresh tube for use or store at -80ºC for future usage.

   Suspension Cells:

   Wash the cells with PBS and pellet the cells by centrifuging. Remove the PBS and add 500ul of 1X RTK Cell Lysis buffer per 10^7 cells and incubate on ice for 10 minutes. Sonicate the cell lysate briefly on ice. Centrifuge the tube for 2 minutes at 10,000 rpm. Transfer the supernatant to a fresh tube for use or store at -80ºC for future usage.

   Tissue:

   For the lysis of tissue samples, extract the tissue by adding 1ml of 2X RTK Cell Lysis buffer per 100mg of tissue. Homogenize the tissue after adding the buffer and incubate on ice for 10 minutes. Sonicate the tissue lysate on ice. Centrifuge the tube for 2 minutes at 10,000 rpm. Transfer the supernatant to a fresh tube for use or store at -80ºC for future usage.

2. Add 500ul of lysate to 5ml of 1X Diluent buffer.
3. The plate has two sections, columns 1-6 and columns 7-12 for two samples respectively. Add 100ul diluted cell lysate to each well of the section. For the 'blank' well, add 100ul 1X Diluent buffer only. Incubate for 2 hours at room temperature with gentle shaking.
4. Invert the plate over an appropriate container and expel the contents forcibly and wash the plate by adding 200µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Completely remove the liquid after each wash by firmly tapping the plate against clean paper towels.
5. Add 100ul of dilute biotin phosphotyrosine antibody and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash in step 4.
7. Add 100µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash in step 4.
9. Add 100ul substrate and incubate for 20-30 minutes at room temperature. The incubation time can be extended based on the intensity of color reaction.
10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example of standard curve

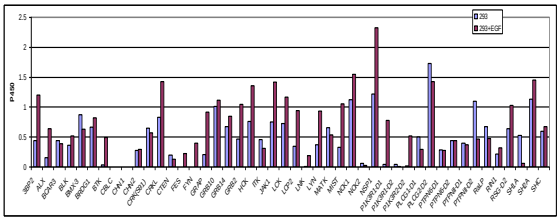

Figure2: Analysis RTK activation with SH2-based RTK profiling assay. HeLa cells were treated with or without EGF 100ng/ml for 5 minutes. The cell lysates were prepared and subjected to the assay.

SH2 Domain Plate Diagram

	1	2	3	4	5	6	1	2	3	4	5	6
A	3BP2	CHN1	GRB10	LNK	PIK3R1-D2	RaLP	3BP2	CHN1	GRB10	LNK	PIK3R1-D2	RaLP
B	ALX	CHN2	GRB14	LYN	PIK3R2-D2	RIN1	ALX	CHN2	GRB14	LYN	PIK3R2-D2	RIN1
C	BCAR3	CRK	GRB2	MATK	PLCG1-D1	RASA1	BCAR3	CRK	GRB2	MATK	PLCG1-D1	RASA1
D	BLK	CRKL	HCK	MIST	PLCG1-D2	SH1A	BLK	CRKL	HCK	MIST	PLCG1-D2	SH1A
E	BMX-3	CTEN	ITK	NCK1	PTPN6-D1	SH2A	BMX-3	CTEN	ITK	NCK1	PTPN6-D1	SH2A
F	BRDG1	FES	JAK1	NCK2	PTPN6-D2	SHC	BRDG1	FES	JAK1	NCK2	PTPN6-D2	SHC
G	BTK	FYN	LCL	NSP1	PTPNII-D1	GST	BTK	FYN	LCL	NSP1	PTPNII-D1	GST
H	CBLC	GRAP	LCP2	PIK3R1-D1	PTPNII-D2	Blank	CBLC	GRAP	LCP2	PIK3R1-D1	PTPNII-D2	Blank