



SH2 Domain-Based RTK Profiling Kit

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.

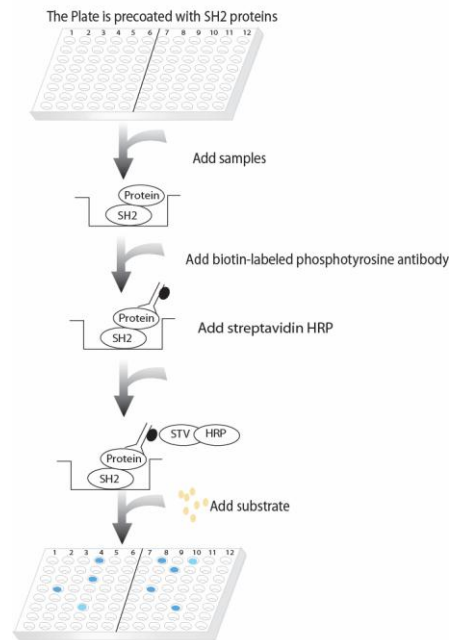


Diagram of SH2 domain-based RTK profiling

Materials provided with the kit

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

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
- 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 2-5x10⁶ cells are plated with the complete medium on 10cm² 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. Wash the cells with PBS and aspirate the PBS **completely**. (Note: Any PBS residue may affect cell lysis process).
2. Add 600ul of 1X Cell Lysis buffer, scrape the cells and collect to 1.5 ml tube and sonicate briefly on ice.
3. Centrifuge the tube for 2 minute at 10,000 rpm. Transfer the supernatant to a fresh tube, and keep the tube on ice. This cell lysate is ready for the following assay or stored at -80°C for the future usage.
4. Dilute 500ul of cell lysate to 5 ml 1X Diluent buffer.
5. Remove the sealing film. The plate has two sections, 1-6 and 7-12 columns for two samples respectively.
6. Add 100ul diluted cell lysate buffer to each well of the section. The 'blank' well add 1X Diluent buffer only. Incubate for 2 hours at room temperature with gently shaking.
7. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 200ul of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

8. Add 100ul of dilute biotin labeled phosphotyrosine antibody, and incubate for 1 hour at room temperature with gentle shaking.
9. Repeat the aspiration/wash in step 7.
10. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
11. Repeat the aspiration/wash in step 7.
12. Add 100ul substrate, and incubate for 10-20 minutes.
13. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
14. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example of standard curve

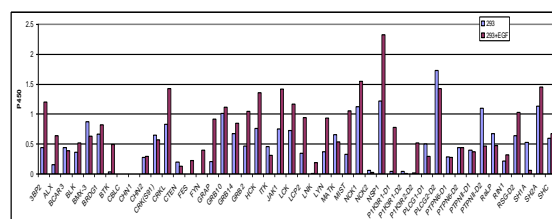


Figure2. Analysis RTK activation with SH2-based RTK profiling assay. HeLa cells were treated with or without EGF 100ng/ml for 5 minutes. The cell lysates were prepared and subjected to the assay.

SH2 Domain Plate Diagram

	1	2	3	4	5	6	1	2	3	4	5	6
A	3BP2	CHN1	GRB10	LNK	P1K3R1-D2	RaLP	3BP2	CHN1	GRB10	LNK	P1K3R1-D2	RaLP
B	ALX	CHN2	GRB14	LYN	P1K3R2-D2	RIN1	ALX	CHN2	GRB14	LYN	P1K3R2-D2	RIN1
C	BCAR3	CRK	GRB2	MATK	PLCG1-D1	RSG	BCAR3	CRK	GRB2	MATK	PLCG1-D1	RSG
D	BLK	CRKL	HCK	MIST	PLCG2-D2	SH1A	BLK	CRKL	HCK	MIST	PLCG2-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	LCK	NSP1	PTPN11-D1	GST	BTK	FYN	LCK	NSP1	PTPN11-D1	GST
H	CBLC	GRAP	LCP2	P1K3R1-D1	PTPN11-D2	Blank	CBLC	GRAP	LCP2	P1K3R1-D1	PTPN11-D2	Blank