SnapCHIP™

Akt
Pathway Activation

Cellular Signaling Array 1

Semi-Quantitative measurement of:
9 total and phospho proteins

Catalog #: PBA-SIG01

User Manual

Last revised Sept 23th, 2018

PARALLEX BIOASSAYS

www.parallexbio.com

I. Table of Contents

II. Overview

Proteins detected (11)	Akt-1	Akt-1 P-ser473
	Akt-2	Akt-2 P-ser474
	Akt-3	Akt-3 P-ser472
		Akt P-Thr308
	S6	S6 P-Ser235/236
	mTOR	mTOR P-Ser
Format	One standard glass slide is spotted with 24 wells of identical antibody arrays. Each antibody is arrayed as 8 replicates.	
Detection Method	Fluorescence 647	
Sample Volume	50 - 100 µL per sub-array	
Specificity	Mouse and Human (Akt-3: mouse preferred)	
Reproducibility	CV <15%	
Assay Duration	6 hours (~1h hands-on time)	

III. Introduction

Multiplexed sandwich immunoassay is a powerful technique to measure multiple protein concentrations simultaneously. Despite a great initial excitement, its success was hampered by cross-reaction, which results in false positive signals. Cross-reaction occurs because the detection antibodies are mixed together when applied to the array. Moreover, it is often impossible to combine related analytes in the same assay due to incompatibility.

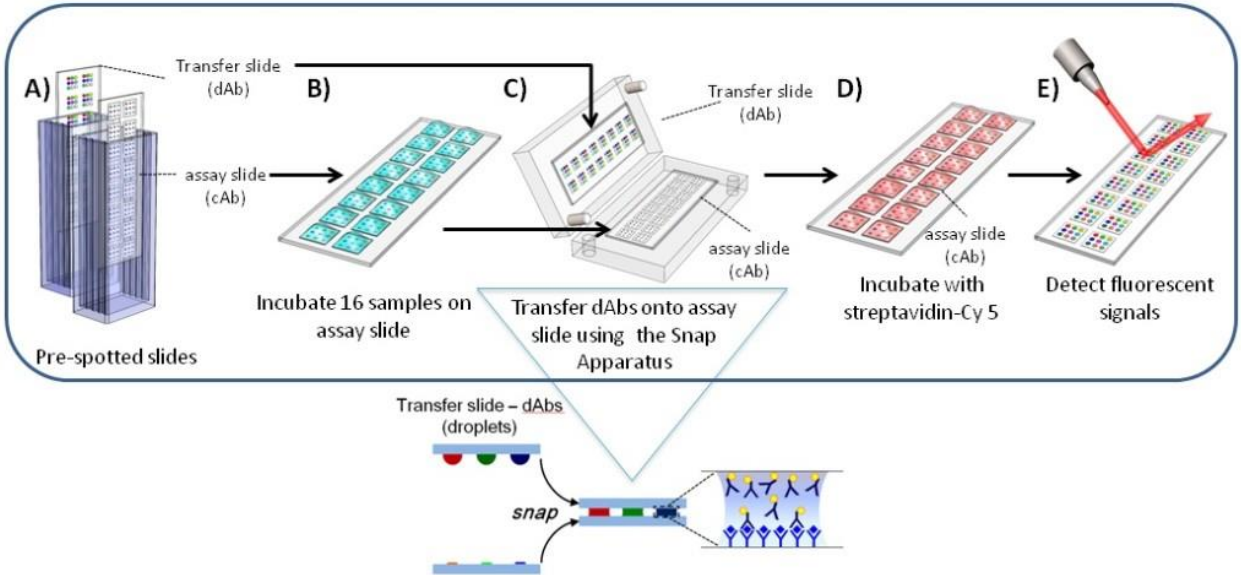
The SnapChip™ developed by Parallex BioAssays is an innovative and simple microarray-to-microarray approach that eliminates the need for mixing the detection antibodies. The absence of cross-reaction is highly desirable to reduce assay development time and cost, ensure accurate results and open ways to new opportunities. The colocalization of the capture and detection antibodies in nanodroplets reproduces the conditions of the common ELISA. It's performed on standard planar arrays, representing an attractive solution for scientists to accelerate their research without investing in expensive equipment. Therefore, it combines the advantages of the high detection sensitivity and specificity of ELISA and the high throughput of arrays. This is a MULTIPLEX assay without compromise. This is a PARALLEX assay.

The SnapChip™ Cellular Signaling Series enables researchers to accurately determine simultaneously the concentration of specific isoforms and phosphorylation state of multiple signaling proteins.

IV. How It Works

The assay process flow using the SnapChip™ is highly similar to ELISA or regular multiplexed sandwich assay on a planar microarray. The assay slide is precisely spotted with capture antibodies that bind to the glass surface. After incubation with your samples, the target proteins are trapped to their respective antibody spots. Following washing steps, biotinylated detection antibodies contained in nanodroplets (on the transfer slide) are precisely delivered to their cognate spots using the SnapDevice™. It is followed by incubation to allow the antibody-antigen intereaction to occur. Finally, after washing, the assay signal is created by an incubation with fluorescent-labelled streptavidin and signals are revealed using a scanner with fluorescent capability.

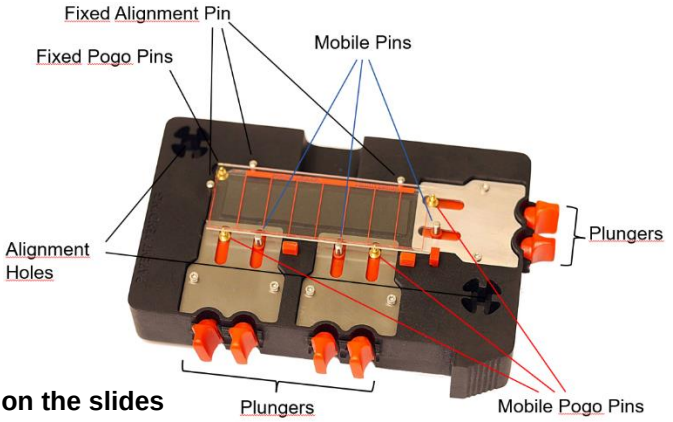
chip2chip technology™



***SnapChip™ assay process flow*. A)** Customers will buy a reusable Snap Apparatus and consumable pre-spotted slides. **B)** They will incubate their samples for 1h on the assay slide harboring the capture antibodies. **C)** After washing and drying the assay slide, they will precisely deliver the biotinylated detection antibodies on their cognate spots using the Snap Apparatus. ▼The slides are brought close to each other to allow the droplets on the transfer slide to bridge with the assay slide and leave liquid behind after separation. A spacer is used to control the distance between the two slides. **D)** After proper incubation and washing, the assay signal will be created by an incubation with Cy5-labelled streptavidin and **E)** fluorescent signals revealed by a scanner with fluorescent capability.

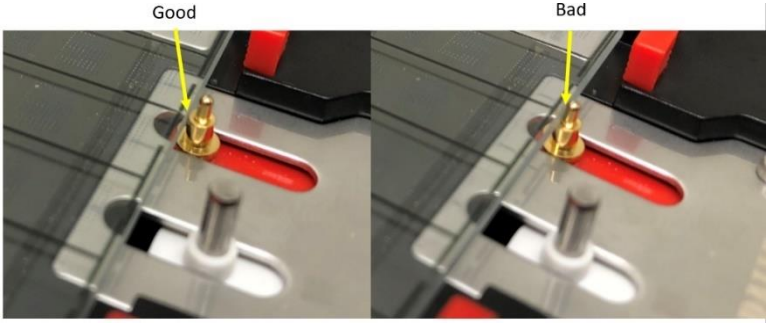
V. SnapDevice™

SnapDevice™ components

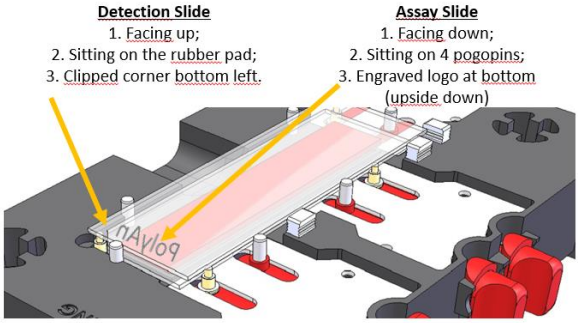


Position the slides

1. Pull and rotate all plungers by 90° to lock them in the open position
2. Position the detection slide into the holder
3. Release the pogo pin plungers in order to push the detection slide against the fixed alignment pins
    a. Note: Be sure the slide does not sit on top of the pogo pin's body (see below)



4. Assay slide is put in place upside down, sitting on the four pogo pins
5. Release the pin plungers in order to push the assay slide against the fixed alignment pins



SNAP!

1. Putting the top shell in place, using the alignment holes.
    a. Don't apply pressure
2. Insert the SnapDevice into its Cage
    a. KEEP all components flat on the bench
    b. Pull up the closure tab
    c. Slide it completely inside the Cage

3. Push down the closure tab to compress the pogo pin and bring the slides together. Wait 10 sec.
4. Take the assay slide back for further processing.

VI. Materials Provided

Catalog #: PBA-DBT01

- Assays slide
- Detection slide (the one with a clipped corner)
- 24 wells gaskets
- Blocking Buffer / Sample Diluent (25 mL)
- Wash Buffer (60 mL)
- Streptavidin-647

VII. Other Supplies Required

- Benchtop rocker or orbital rocker
- Microarray centrifuge (or compressed air/gas)
- Laser scanner for fluorescence detection
- 1.5 mL Polypropylene microcentrifuge tubes
- Slide washing plate (cat.no. PBA-WP04)
- 60% humidity chamber (cat.no. PBA-HU60)

VIII. Storage

The SnapChip™ kit should be kept at 4°C. It will retain its activity for up to 6 months following purchase.

IX. General Considerations

Sample Preparation

- Prepare your tissues of cells lysates with buffer containing protease and phosphatase inhibitors.
- Determine the concentration of protein for each sample (*e.g.* BCA of Bradford)
- We recommend to experimentally determine the protein concentration that should be used to ensure results fall in the linear portion. To do so, run a representative sample at different concentration (1:2 dilution series).

Handling Glass Slides

- Do not touch the surface of the slides, as both assay and detection slides are very sensitive. Hold the slides by the edges only.
- Handle glass slides in clean environment with powder free gloves.
- Red and black permanent markers can significantly interfere with fluorescent signal detection. We recommend marking your slides with a green or blue ultra-fine point permanent marker. Do not write on the arrayed well areas.

Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- In order to prevent evaporation, cover the incubation chamber with an adhesive film during >30 min incubations.
- Sample incubation may be done either at room temperature for 2h or overnight at 4°C.

X. Reagents Preparation

- Streptavidin-conjugate : Add 10mL of blocking buffer

XI. Protocol

A. Antigen Incubation

A.1. Take the slides out from the fridge, and let the package to stand at room temperature for 30 min before opening.
A.2. Add 80µL of Blocking buffer in each well.
A.3. Incubate @ RT for 20min on the rocking plate @ 450 RPM.
A.4. Dilute your samples with the sample diluent buffer.
A.5. Discard the blocking from the well by slapping on an absorbent paper.
A.6. Add your samples (50 to 100µL).
A.7. Incubate @ RT for 2h on the rocking plate @ 450 RPM.
A.8. Rince 3x 5 minutes with wash buffer and disassemble the gasket
A.9. Put the Assay slide in the slide washing plate and wash 1 x 1 minutes with 5mL of ddH2O at room temperature, on a rocking plate at 450RPM.
A.10. Dry the slide using a microarray centrifuge or a nitrogen stream.

B. SNAP

For more detailed information, also refer to section V and the video accessible online @ www.parallexbio.com

B.1. Incubate the Transfer slide 30min @ ambient humidity.
B.2. Pull all plungers on the SnapDevice™.
B.3. Insert the Detection slide and release the pogo pin plungers
B.4. Insert the Assay slide and release the pin plungers.
B.5. Gently assemble the Top Shell.
B.6. Lift the Closure Tab, and insert the Device into its Cage.
B.7. Push the Closure Tab down and wait 10 sec.
B.8. Take the Assays slide out, and incubate it 1h @ 60% humidity. Discard the Transfer slide.

C. Get the Assay Signals

C.1. Put the Assay slide in the slide washing tray and wash 3 x 5 minutes with 5mL of wash buffer at room temperature, on a rocking plate at 450RPM.
C.2. Add the Streptavidin-conjugate.
C.3. Incubate @ RT for 30min on the rocking plate @ 450 RPM
C.4. Wash 3 x 5 minutes with 5mL of wash buffer at room temperature, on a rocking plate at 450RPM.
C.5. Rinse with ddH_2O and dry the slide using a microarray centrifuge or a nitrogen stream.
C.6. Scan (Ex650Em665)
(Please contact us for a free scanning service)

XII. Array Map

Each assay slide is divided into 24 wells of identical antibody sub-arrays composed of 8-replicates rows. Positive controls (+) are positioned on column 9.

	1	2	3	4	5	6	7	8	9
A	Akt-1								(+)[1]
B	AKt-1 Phospho Ser473								(+)[2]
C	Akt-2								(+)[1]
D	AKt-2 Phospho Ser474								(+)[2]
E	Akt-3								(+)[1]
F	AKt-3 Phospho Ser472								(+)[2]
G	Akt Phospho Thr308								(+)[1]
H	S6								(+)[2]
I	S6 Phospho Ser								(+)[1]
J	mTOR								(+)[2]
K	mTOR Phospho Ser235/6								(+)[1]
L									(+)[2]

XIII. Data Analysis

Fluorescent microarray scanner is required. The fully processed microarrays can be sent to Parallex BioAssays for scanning at no charge using the red slide mailers provided.

Images from scanned microarrays can be analysed with your scanner-associated software or others commercially and/or freely available image analysis softwares.

Parallex BioAssays provide an Excel file for experiment record and rapid data analysis. It is mainly prefilled, easy to use for a rapid basic analysis and generating graphs. Please visit our website (www.parallexbio.com) to download the file suited to your kit.

XIV. Troubleshooting Guide

Please refer to the following problems, causes and recommendations.

Weak Signal

1. Inadequate detection
   - Adjust the scanning parameters (Increase laser power and/or PMT)
2. Short incubation time
   - Increase incubation time or change sample incubation step to overnight
3. Too low protein concentration in sample
   - Lessen dilution or do not dilute sample
   - Concentrate sample if necessary
4. Improper storage
   - Store kit as suggested temperature. Don't freeze/thaw the slide.
   - Always use fresh samples (or properly stored) prepared with protease and phosphatase inhibitors.

Uneven signal

5. Bubble formed during incubation
   - Decrease amount of rocking during incubations
   - Check for bubble formation and remove bubbles
6. Arrays are not completed covered by reagent
   - Completely cover arrays with solution for all required steps
7. Reagent evaporation
   - Cover the incubation chamber with adhesive film during incubation
8. Comet tail formation
   - Air dry the slide for at least 1 hour before usage

High background

9. Cross-contamination from neighboring wells
   - Avoid overflowing wash buffer and other solutions into neighboring wells.
10. Overexposure / saturated signals
    - Adjust the scanning parameters (decrease laser power and/or PMT)
    - Further dilute your samples
11. Insufficient wash
    - Completely remove wash buffer in each wash step
    - Increase wash time and use more wash buffer
12. Dust and/or auto-fluorescent debris
    - Work in a clean environment

XV. Publications

1. H. Li, S. Bergeron, H. Larkin and D. Juncker. Snap chip for cross-reactivity-free and spotter-free multiplexed sandwich immunoassays. ***J. Vis. Exp.*** (129), e56230, doi:10.3791/56230 (2017).
2. H. Li, S. Bergeron, M. Annis, P. Siegel and D. Juncker. Serial analysis of 38 proteins during the progression of human breast tumor in mice using an antibody colocalization microarray. **Mol Cell Proteomics**. 2015 Apr;14(4):1024-37
3. D. Juncker, S. Bergeron, V. Laforte and H. Li. *Cross-reactivity in antibody microarrays: shedding light on the dark side of multiplexing (review)*. **Current Opinion in Chemical Biology**. 2014, 18:29-37.
4. Li H, Bergeron S and Juncker D. *Microarray-to-microarray transfer of reagents by snapping of two chips for cross-reactivity-free multiplex immunoassays.* **Analytical Chemistry.** 2012 April.
5. M. Pla-Roca, R. F. Leulmi, S. Tourekhanova, S. Bergeron, E. Moreau, V. Laforte, S. Goselin, N. Bertos, M. Hallett, M. Park, D. Juncker. *Antibody Colocalization Microarrays: A scalable platform for protein analysis in complex samples*. **Molecular and Cellular Proteomics**. 2012.

To learn more about the SnapChip® Technology, visit www.parallexbio.com

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