Rapid Akt Pathway Profiling Using the SnapChip™

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Introduction

Akt activation orchestrates diverse biological processes, such as proliferation, survival and glucose metabolism. Its action is mediated by a phosphorylation cascade and the activation of downstream proteins, including mTOR and S6 (Fig. 1). Akt exists in 3 isoforms (Akt-1, Akt-2 and Akt-3) which have both overlapping and distinct roles[5], so looking at it as a unique protein may lead to misleading results. The SnapChip™ (cat.no. PBA-SC001) offers a unique combination of assays, thanks to the absence of cross-reaction, to rapidly get an overview on the activation status of the Akt pathway, with information on specific isoforms and phosphorylation sites.

In this technical note, we show results obtained with mouse liver and white adipose tissue (WAT) lysates. We demonstrate the importance of the sample's total protein concentration to make sure the measurements fall into the dynamic range of the assay. Moreover, we show how the SnapChip™ can be used to evaluate the insulin sensitivity in your mouse & human studies.

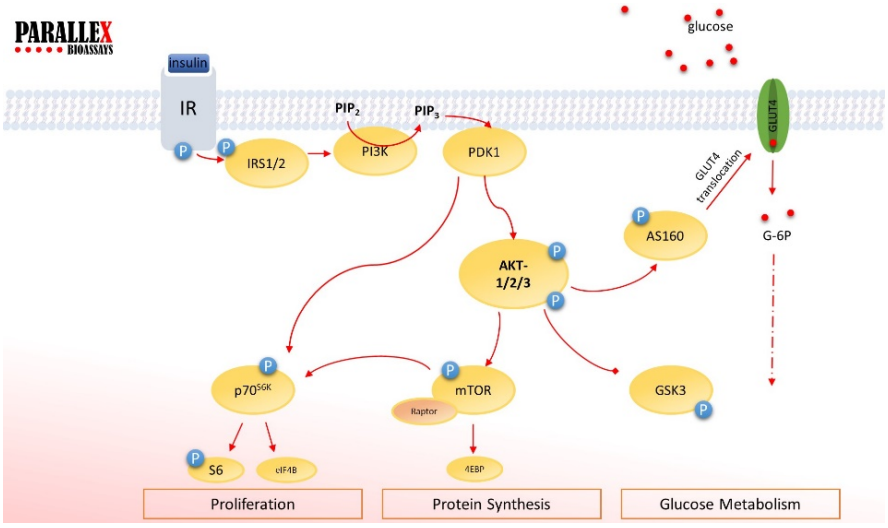

Figure 1: The activation of the Akt pathway through a phosphorylation cascade has a central role in many biological processes, including proliferation, protein synthesis and glucose metabolism.

Methods

Animals: Animal handling and treatment were approved by the Animal Care and Handling Committee of Laval University. Mice were fasted for 5 h and injected intraperitoneally with saline or regular human insulin (1.5 U/kg) 10 min before the sacrifice. Liver and epididymal fat pad (WAT) were removed and immediately frozen in liquid nitrogen and stored at ±80°C until further analysis.

Tissue Lysates: Frozen tissues were pulverized with a mortar and pestle in liquid nitrogen, and the powder was homogenized in 3-5 volumes of lysis buffer (PBS supplemented with 1% NP-40, 10 mM NaF, 2 mM Na_3VO_4, 1 mM PMSF, and cOmplete™ protease inhibitors (Roche)). Cell debris of insoluble materials was removed by centrifugation at 14,000 ×g for 10 min. Total protein concentration was determined using BCA protein assay reagents (Pierce).

SnapChip™ Assays: The SnapChip™ protocol is very similar to standard ELISA and other planar arrays. The only difference is the use of the SnapDevice™ to precisely deliver the detection antibodies as an array of nanodroplets, which requires 5 min of training and less than 2 min to perform. Please refer to our website for a complete user manual, additional information, and videos (www.parallexbio.com/resources).

Results

When measuring protein concentrations, regardless of the method used, sample dilution is an important consideration. Samples that are too dilute will not show any signal for the protein of interest, while the signal will saturate for samples that are too concentrated. To sense variations in protein concentration induced by your treatments, the concentration of your protein of interest should fall in the dynamic range of the assay. The dynamic range can be determined by running a dilution series of a representative sample on the SnapChip™. Based on the results presented on figure 2, when working with liver lysates of mouse origin, we recommend a total protein concentration between 0.5 and 2 mg/mL. More information can be found in the user manual of your assay kit.

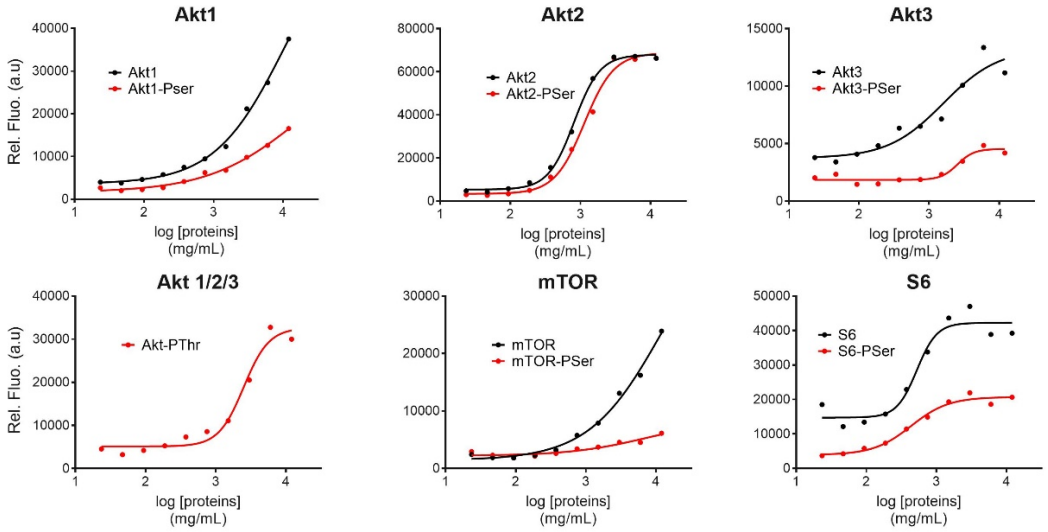

Figure 2. **Dilution series.** Liver lysate from an insulin-treated mouse was diluted from 12 mg/mL to 0.023 mg/mL in a 2-fold dilution series. The SnapChip™ was used to detect Akt1 (total and P-ser473), Akt2 (total and P-ser474), Akt3 (total and P-ser472), Akt P-thr308, mTOR (total and P-ser2481) and S6 (total and P-ser235/236) in a single experiment.

The SnapChip™ can be used to rapidly assess the effect of treatments or diets on insulin sensitivity at the cellular level via an Akt pathway profile. In this Technical Note, we looked at the effect of insulin stimulation in the WAT of control mice, compared to saline-treated mice (Fig. 3). We detected a significant increase in the

phosphorylation of both serine and threonine residues of Akt. Of note, there is a more potent activation of the Akt-2 isoform which is aligned with the conclusions of other studies[5].

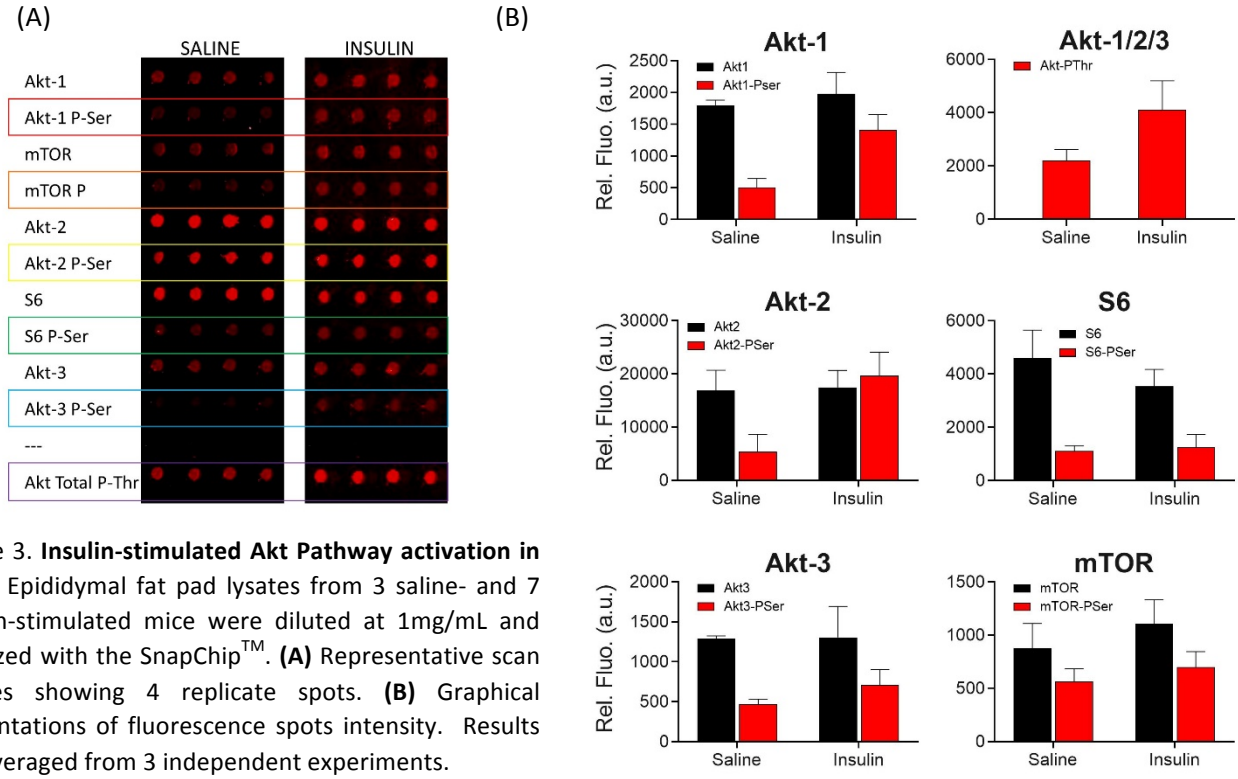

Figure 3. **Insulin-stimulated Akt Pathway activation in WAT.** Epididymal fat pad lysates from 3 saline- and 7 insulin-stimulated mice were diluted at 1mg/mL and analyzed with the SnapChip™. **(A)** Representative scan images showing 4 replicate spots. **(B)** Graphical presentations of fluorescence spots intensity. Results are averaged from 3 independent experiments.

The SnapChip™ used in this publication (cat.no. PBA-SC001) is a semi-quantitative assay. Simple data analysis allows the comparison of the protein expression and the level of phosphorylation among the samples tested, which should be taken into consideration when interpreting the results. As recommended for Western Blot[6], it is possible to use a dilution series of a pool or a representative sample to generate a standard curve and evaluate more accurately the variations in the concentration and the level of phosphorylation of the target proteins.

Conclusion

The SnapChip™ was used to measure key proteins and PTM in the Akt pathway. The colocalization of the capture and detection antibodies allows to mix-and-match any existing singleplex. Indeed, the analysis of the 3 Akt isoforms in the same assay is not possible with other multiplex technologies because of cross-reactions and a lack of specificity of the antibodies. On the SnapChip™, the assays are contained in nanodroplets and are physically isolated, which opens new opportunities and eliminates the risk of false positives. Other multiplex assays are available, and customized chips can be rapidly developed.

References:
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