
# Easy50™ PI3K Inhibitor Array*

**Catalog # IAP002**

# Instruction Manual

**Please read all instructions carefully before use**

**For Research Use Only**

***Patent pending**

**www.symansis.com**

## Introduction

This Easy50™ PI3K Inhibitor Array is a panel of inhibitors that are known to specifically target the PI3K signaling pathway, and related pathways. Data generated with the Easy50™ array produces an inhibition profile that provides a more comprehensive understanding of the specific PI3K signaling pathway involved in a particular response.

These inhibitors are provided in a 96-well plate, and are simply dissolved in DMSO, and then diluted in cell culture medium and added to cells, or diluted in buffer for kinase assays.

## Materials provided

1. One 96-well microplate containing inhibitors as shown in the **Plate setup**. The microplate is sealed under argon with aluminium sealing foil.
2. One plate cover to seal plate while inhibitors are dissolving in DMSO.
3. 12 strips of caps to cover the wells of the microplate once inhibitors are dissolved in DMSO.

## Materials required but not provided

DMSO (dimethyl sulfoxide), approximately 3.5 ml.

## Storage and stability

Store microplate at ambient temperature (<25°C) and use within 1 year. After re-suspension of the inhibitors in DMSO, seal and store at -20° in the foil pouch provided, and use within 3 months. Avoid repeated freeze-thaw cycles. Hygroscopic. Protect from light.

## Safety precautions

Avoid contact with skin. Wear appropriate personal protection (labcoat, gloves, safety glasses). For specific safety instructions, refer to the material safety data sheet provided.

## Directions for use

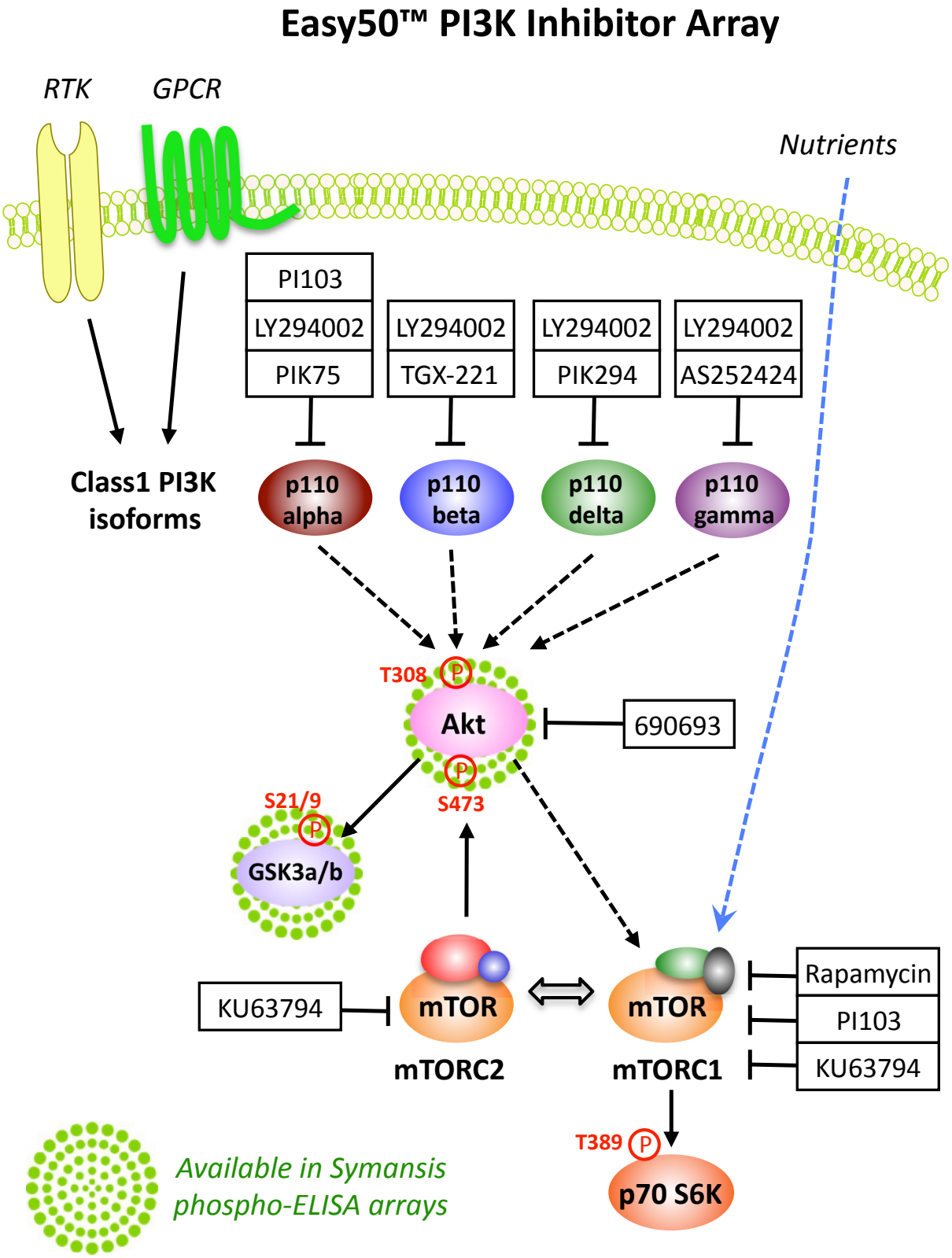
see page 5

## Data interpretation

To determine the $EC_{50}$ construct a graph of the cellular response you are measuring versus the diluted drug concentration. The drug concentration at which the response is inhibited 50% is the $EC_{50}$.

If the $EC_{50}$ correlates closely with $IC_{50}$ (see **Inhibitor information** for $IC_{50}$ values) then this provides evidence that the enzyme targeted by the drug is required for the cellular process you measure.

The diagram below indicates which intermediate proteins are targeted by the various inhibitors. Compare the inhibition profile obtained from a screening with the diagram to identify the probable intermediate proteins in the PI3K pathway involved in a particular response.



## Plate setup

The following table shows the concentrations of each inhibitor after re-suspension in 35 µl of DMSO ( = stock solution).
The recommended final or working concentration is 1/1000 of the stock solution.

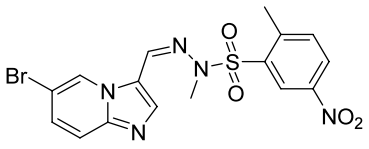
| **Target:** | **p110α (alpha)** | **p110β (beta)** | **p110δ (delta)** | **p110α p110β** | **p110α p110δ** | **p110α p110β p110δ** | **p110γ (gamma)** | **PI3K** | **PI3K DNA-PK mTOR** | **mTOR** | **mTOR** | **Akt** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Inhibitor:** | **PIK75** | **TGX-221** | **PIK294** | **PIK75** + **TGX-221** | **PIK75** + **PIK294** | **PIK75** + **TGX-221** + **PIK294** | **AS252424** | **LY294002** | **PI-103** | **Rapamycin** | **KU63794** | **690693** |
| **Well #** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | 1 mM | 2.5 mM | 1 mM | (dilutions as for single inhibitor) | | | 10 mM | 50 mM | 1 mM | 5 mM | 1 mM | 5 mM |
| **B** | 500 µM | 1 mM | 500 µM | (dilutions as for single inhibitor) | | | 5 mM | 10 mM | 500 µM | 1 mM | 500 µM | 1 mM |
| **C** | 100 µM | 500 µM | 100 µM | (dilutions as for single inhibitor) | | | 1 mM | 5 mM | 100 µM | 500 µM | 100 µM | 500 µM |
| **D** | 50 µM | 100 µM | 50 µM | (dilutions as for single inhibitor) | | | 500 µM | 1 mM | 50 µM | 100 µM | 50 µM | 100 µM |
| **E** | 10 µM | 50 µM | 10 µM | (dilutions as for single inhibitor) | | | 100 µM | 500 µM | 10 µM | 50 µM | 10 µM | 50 µM |
| **F** | 5 µM | 10 µM | 5 µM | (dilutions as for single inhibitor) | | | 50 µM | 100 µM | 5 µM | 10 µM | 5 µM | 10 µM |
| **G** | 1 µM | 5 µM | 1 µM | (dilutions as for single inhibitor) | | | 10 µM | 50 µM | 1 µM | 5 µM | 1 µM | 5 µM |
| **H** | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO | DMSO |

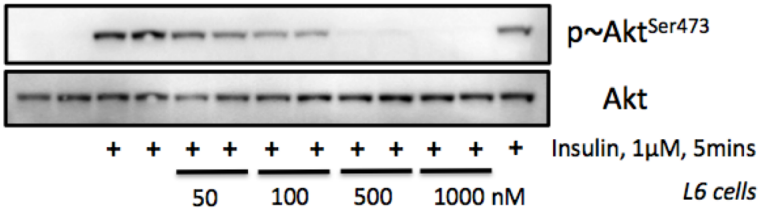
## Directions for use

- Open the pouch and remove the microplate. Orientate the plate as shown in **Plate setup** and carefully remove the sealing foil.
- Add 35 µl of DMSO to each well. (35 µl will achieve the stock concentrations shown in the **Plate setup**).
- Cover plate with sealing film provided, then gently dissolve inhibitors by placing the plate on a horizontal orbital microplate shaker set at low speed (400 rpm) for 5 minutes. Alternatively, dissolve by pipetting 5x (10-20x for LY294002).
- Remove sealing film, and cap any unused columns using capstrips.
- Pipette appropriate volumes of inhibitor stock solutions into cell culture medium or kinase buffer. A 1/1000 dilution is required (ie. for 2 ml of working solution, add 2 µl of inhibitor stock solution).
- Ensure that the inhibitor stock solution is fully dissolved in medium / buffer. If the inhibitor solution is pipetted directly into cell culture wells, mix by moving the plate gently to swirl the medium around the well.
- The appropriate incubation time of inhibitors with cells or enzymes should be determined by the investigator depending on their research needs. As a guide, the Easy50 PI3K array inhibitors have been tested with a 10-minute incubation time before stimulus of signaling pathways.
- The bottom well of each column of an inhibitor is empty, and can be used as an experimental control by adding DMSO as for the other wells.
- After use, cap the opened wells with the cap-strips provided, pressing firmly to seal. Return the plate to the foil pouch, and store at -20°.

## Inhibitor information

| **PIK75** | **CAS Number** [372196-67-3] | **Chemical Formula:** $C_{16}H_{14}BrN_5O_4S$ | **Molecular Weight:** 452.28 |
|---|---|---|---|



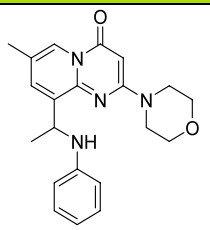



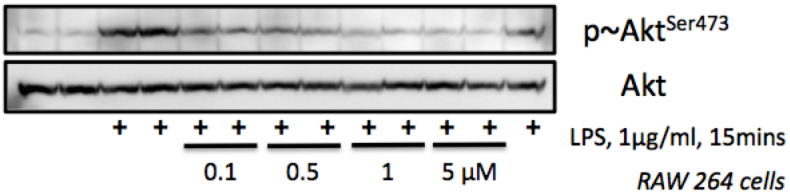

**Background information**

PI3K alpha-selective inhibitor with an in vitro $IC_{50}$ of 8 nM for p110 alpha, 343 nM for p110 delta, and 907 nM for p110 beta[1].

1. Chaussade C, et al. (2007) Biochem J. 404(3):449-58. Evidence for functional redundancy of class IA PI3K isoforms in insulin signalling.

| TGX221 | CAS Number [663619-89-4] | Chemical Formula: $C_{21}H_{24}N_4O_2$ | Molecular Weight: 364.44 |
|---|---|---|---|



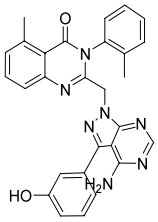


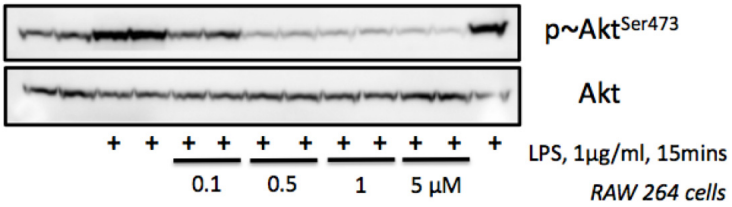

**Background information**

PI3K beta-selective inhibitor with an in vitro $IC_{50}$ of 9 nM for p110 beta, 211 nM for p110 delta, and >1000 nM for p110 alpha[1].

1. Chaussade C, et al. (2007) Biochem J. 404(3):449-58. Evidence for functional redundancy of class IA PI3K isoforms in insulin signalling.

| PIK294 | CAS Number [900185-02-6] | Chemical Formula: $C_{28}H_{23}N_7O_2$ | Molecular Weight: 489.53 |
|---|---|---|---|






**Background information**

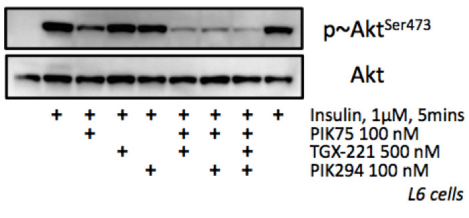
PI3K delta-selective inhibitor with an in vitro $IC_{50}$ of 10 nM for p110 delta, 160 nM for p110 gamma, 490 nM for p110 beta, and 10 µM for p110 alpha[1].

1. Knight ZA, et al. (2006) Cell. 125(4):733-47. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling.

## PIK75 / TGX221 / PIK294

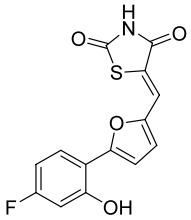
Evidence suggests that p110 isoforms can be functionally redundant (ref 1 and data at right). The use of combinations of alpha, beta, and delta inhibitors facilitates testing of compensation by one isoform for another.

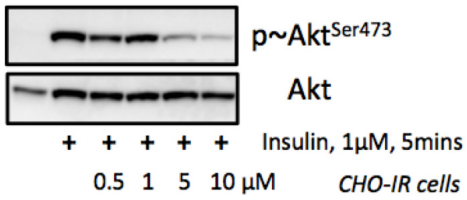
1. Chaussade C, et al. (2007) Biochem J. 404(3):449-58. Evidence for functional redundancy of class IA PI3K isoforms in insulin signalling.



Note: PIK75, TXG221, PIK294, AS252424, PI103, KU0063794 and 690693 are also available from Symansis in 1-100 mg quantities.

| AS-252424 | CAS Number | Chemical Formula: | Molecular Weight: |
|---|---|---|---|
| | [900515-16-4] | $C_{14}H_8FNO_4S$ | 305.28 |



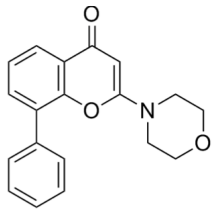


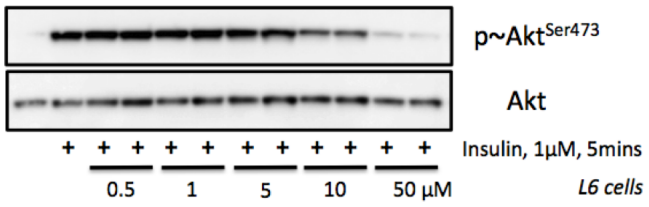

**Background information**

PI3K gamma-selective inhibitor with an in vitro $IC_{50}$ of 30 nM for p110 gamma, 940 nM for p110 alpha, and >20 µM for p110 beta and delta[1].

1. Pomel V, et al. (2006) J Med Chem. 49(13):3857-71. Furan-2-ylmethylene thiazolidinediones as novel, potent, and selective inhibitors of phosphoinositide 3-kinase gamma.

| LY-294002 | CAS Number | Chemical Formula: | Molecular Weight: |
|---|---|---|---|
| | [154447-36-6] | $C_{19}H_{17}NO_3$ | 307.34 |



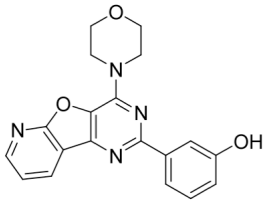


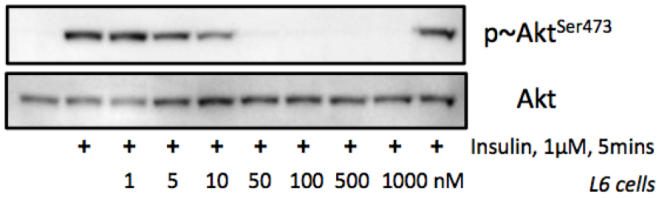

**Background information**

PI3K inhibitor with an in vitro $IC_{50}$ of 500 nM for p110 alpha, 570 nM for p110 delta, and 973 nM for p110 beta[1].

1. Chaussade C, et al. (2007) Biochem J. 404(3):449-58. Evidence for functional redundancy of class IA PI3K isoforms in insulin signalling.

| PI-103 | CAS Number | Chemical Formula: | Molecular Weight: |
|---|---|---|---|
| | [371935-74-9] | $C_{19}H_{16}N_4O_3$ | 348.36 |



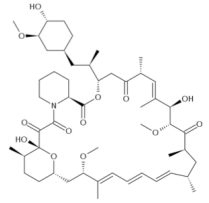


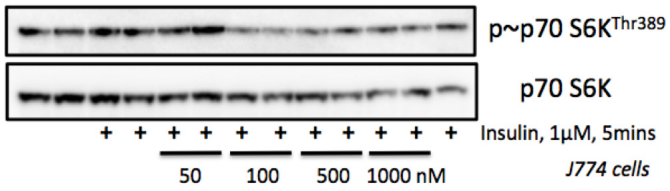

**Background information**

A kinase inhibitor with an in vitro $IC_{50}$ of 2 nM for DNA-PK, 8 nM for p110 alpha (PI3K), and 20 nM for mTORC1[1].

1. Knight ZA, et al. (2006) Cell. 125(4):733-47. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling.

| Rapamycin | CAS Number [53123-88-9] | Chemical Formula: $C_{51}H_{79}NO_{13}$ | Molecular Weight: 914.17 |
|---|---|---|---|



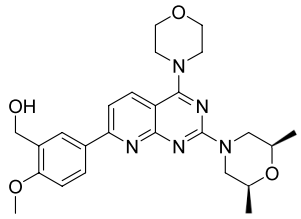


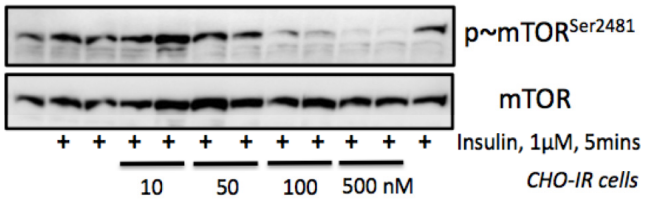

**Background information**

Immunosuppressant drug and inhibitor of mammalian-Target-Of-Rapamycin, with an in vitro $IC_{50}$ of <10 nM for mTORC1[1].

1. Guertin, DA and Sabatini, DM (2009) Sci. Signal. 2 (76) pe2. The Pharmacology of mTOR inhibition.

| KU0063794 | CAS Number [938440-64-3] | Chemical Formula: $C_{25}H_{31}N_5O_4$ | Molecular Weight: 465.54 |
|---|---|---|---|



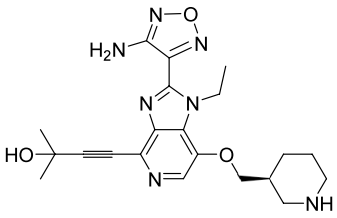


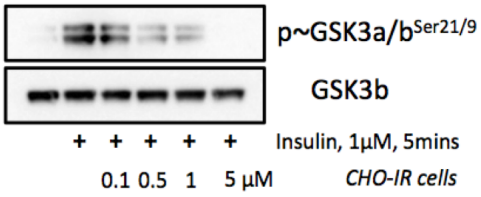

**Background information**

Inhibitor of mammalian-Target-Of-Rapamycin complexes, with an in vitro $IC_{50}$ of 10 nM for mTORC1 and mTORC2[1].

1. García-Martínez, JM et al. (2009) Biochem. J 421: 29–42. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR).

| 690693 | CAS Number [937174-76-0] | Chemical Formula: $C_{21}H_{27}N_7O_3$ | Molecular Weight: 425.48 |
|---|---|---|---|






**Background information**

Pan-Akt kinase inhibitor with an in vitro $IC_{50}$ of 2nM for Akt1, 13 nM for Akt2, and 9 nM for Akt3[1].

1. Heerding, DA et al. (2008) J. Med. Chem., 51:5663–5679. Identification of 4-[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3S)-3-piperidinylmethyl]oxy}-1H-imidazo[4,5-c]pyridin-4-yl]-2-methyl-3-butyn-2-ol (GSK690693), a novel inhibitor of AKT kinase.