Lipid Kinase ADP-Glo Assay Protocol (Manual Assay)

Standard Procedure for Lipid Kinases

Materials:

ADP (e.g. Promega, # V9101)
ADP-Glo™ Reagent (e.g. Promega, # V9101)
ATP, UltraPure (e.g. Promega, # V9101)
CHAPS (e.g. Roth # 1479)
DTT (e.g. Sigma, # D-0632)
EDTA (e.g. Roth, # 8040.1)
EGTA (e.g. Roth # 3054)
H_2O p.a. (e.g. VWR, # 1.16754.9010)
HEPES (e.g. Sigma, #H-3375)
Kinase Detection Buffer (e.g. Promega, # V9101)
Kinase Detection Substrate (e.g. Promega, # V9101)
$MgCl_2$ (e.g. Sigma, # M-3634)
$MnCl_2$ (e.g. VWR, # 1.05927.1000)
PI (L-alpha-Phosphatidylinositol) (e.g. Avanti Polar Lipids # 840044)
PIP2 (8:0 PI(4,5)P2 (1,2-Dioctanoyl-sn-Glycero-3-(Phosphoinositol-4,5-Bisphosphate)) e.g. Avanti Polar Lipids # 850185)
PS (1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-L-Serine]) (e.g. Sigma # 51581)
96-well white flat bottom half area plate (e.g. Corning NBS #3642)

Prepare in advance (store at 4° C):

Lipid-Kinase-Assay-Buffer (2x stock)
- 100 mM HEPES-NaOH, pH 7.5
- 200 mM NaCl
- 0,06 % CHAPS
- 4 mM DTT

Lipid-Kinase-Assay-Buffer (3x stock)
- 150 mM HEPES-NaOH, pH 7.5
- 300 mM NaCl
- 0,09 % CHAPS
- 6 mM DTT

ATP-Dilution-Buffer (with Mg) (10x stock)
- 150 mM $MgCl_2$
- 50 mM EGTA

ATP-Dilution-Buffer (with Mn) (10x stock)
- 150 mM $MnCl_2$
- 50 mM EGTA

Final Assay Conc. *(25 µl reaction cocktail)*:
- 50 mM HEPES-NaOH, pH 7.5
- 3 mM $MgCl_2$ **or** $MnCl_2$
- 1 mM EGTA
- 100 mM NaCl
- 0,03 % CHAPS
- 2 mM DTT
- ATP [var. conc. 0,1 - 500 µM]
- 1 % (v/v) DMSO *(w, w/o test cpd)*
- Substrate (variable)
- Recombinant Lipid Kinase (variable)

Name	Substrate	Add
PI4KB	+PI	+Mn
PI4K2A	+PI:PS	+Mn
PI4K2B	+PI:PS	+Mn
PIK3C2A	+PI	+Mg
PIK3C2B	+PI:P	+Mn
PIK3C2G	+PI:PS	+Mg
PIK3C3	+PI	+Mn
PIK3CA/PIK3R1	+PIP2:PS	+Mg
PIK3CB wt/PIK3R1	+PIP2:PS	+Mg
PIK3CB E633K/PIK3R1	+PIP2:PS	+Mg
PIK3CB E1051K/PIK3R1	+PIP2:PS	+Mg
PIK3CB D1067V/PIK3R1	+PIP2:PS	+Mg
PIK3CD/PIK3R1	+PIP2:PS	+Mg
PIK3CG	+PIP2:PS	+Mg
PIP5K1A	+PI:PS	+Mn
PIP5K1B	+PI:PS	+Mn
PIP5K1C	+PI:PS	+Mn

Manual Assay Procedure:

In white flat bottom half area plate:

A: Activity Assay w/o cpd:

1. Add 10 µl LipidKinase-Assay-Buffer + DMSO [(7.5 µl 2x stock) + 2.5µl 10% DMSO] *(when no cpd is used)*
2. Add 5 µl substrate (in 1x LipidKinase-Assay-Buffer)
3. Add 5 µl recombinant LipidKinase (in 1x LipidKinase-Assay-Buffer)
4. Add 5 µl ATP (in 1x ATP-Dilution-Buffer (Mg or Mn))

B: Inhibitor Assay with cpd.

1. Add 10 µl LipidKinase-Assay-Buffer + ATP [(5 µl 3x stock) + 5µl ATP in 1x ATP-Dilution-Buffer (Mg or Mn)]
2. Add 5 µl 5% DMSO *(w, w/o test cpd)*
3. Add 10 µl of substrate-LipidKinase-Mix (in 1x LipidKinase-Assay-Buffer)
4. ----------
5. Mix on shaker
6. Incubate for 40 min. at 30°C
7. Stop reaction with 25 µl ADP-Glo Reagent
8. Incubate for 40 min. at RT
9. Add 50 µl Kinase Detection Reagent
10. Incubate for 60 min. at RT
11. **Shield plate from light to avoid background!**
12. Count with Luminescence Reader