DEVELOPMENT OF DIACYLGLYCEROL KINASE ASSAYS TO FACILITATE ISOFORM-SPECIFIC INHIBITOR DISCOVERY

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Introduction

Diacylglycerol (DAG) and phosphatidic acid (PA) are two key second messengers in signaling and metabolic pathways. Diacylglycerol kinases (DGK) phosphorylate DAG to produce PA, acting as a central switch between the various signal transduction pathways activated by these second messengers. Ten DGK isoforms (α, β, γ, δ, η, κ, ε, ζ, ι, and θ) have been identified and categorized into five classes based on their structural features.

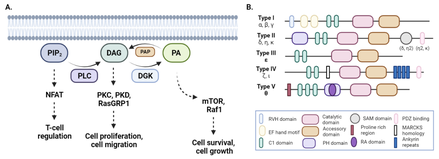


Figure 1: Role of DGK in cell signaling (A), Ten DGK isoforms depicting key functional domains (B).

Inhibition of specific DGK isoforms are therapeutically relevant

- DGKα: highly expressed in melanoma, hepatocellular carcinoma, and glioblastoma cells plays role in cancer cell proliferation and attenuates apoptosis
- DGKα and DGKζ: expressed at high levels in T cells, promotes T cell anergy

DGK isoforms are shown to be associated with several disease conditions such as epilepsy, autoimmunity, cardiac hypertrophy, hypertension, type II diabetes, bipolar disorder (DGKη) and Parkinson's diseases (DGKθ). Thus, isoform-selective DGK inhibition/activation is essential for therapeutics development.

At Reaction Biology Corp., we have developed a DGK profiling panel including all DGK isoforms to provide a tool to facilitate selectivity profiling during DGK inhibitor discovery.

Assay Principle

1. Enzymatic transfer of a phosphate group from ATP to commercial lipid substrate DLG resulting in phosphorylated substrate and ADP.
2. Indirect quantification of ADP via ADP-Glo detection reagent (Promega) by generation of luminescence

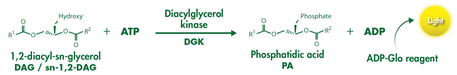


Figure 2: Diacylglycerol kinase reaction.

Assay Optimization

Reaction Biology's DGK assays are optimized by buffer screening with 1,2-dilauroyl-sn-glycerol (DLG) lipid as substrate

Figure 3 shows the buffer optimization using two representative DGK isoforms (DGKα and ζ) in the presence of different detergent and additive combinations. Buffer condition for maximal enzymatic activity was identified based on buffer screening.

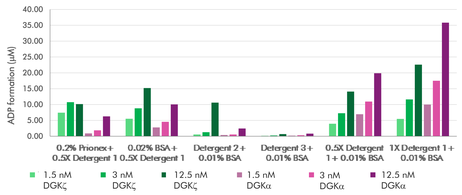


Figure 3: Assay buffer optimization for DGK isoforms.

10 DGK isoforms were titrated at constant ATP and DLG substrate concentrations in order to identify the optimal enzymatic concentration for compound screening applications (Figure 4A, 4B). DGKκ did not show enzymatic activity at this assay condition. In the presence of brain phosphatidylserine (PS) in the assay, enzymatic activity of DGKκ is observed (Figure 4C). DLG substrate concentration in the screening assay is selected to obtain maximal enzymatic activity.

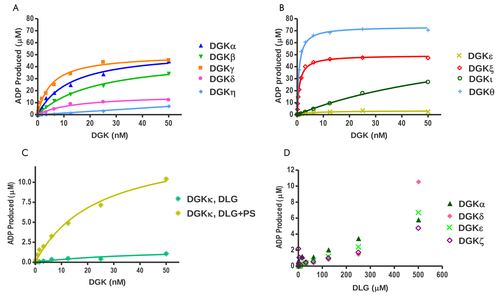
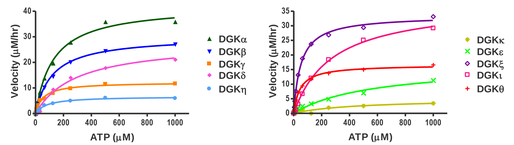


Figure 4: Enzyme titrations, activity comparison of DGK isoforms (A,B), DGKκ enzyme titration at fixed [DLG] +/- PS (C), Enzymatic activity of representative DGK isoforms with increasing [DLG] (D)

Km_{app} Determination and assay validation

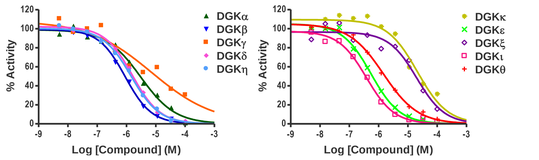
We determined the apparent ATP Km values for each DGK isoforms at 500 μM DLG concentration and using DGK concentrations obtained from titration. The obtained Km, Vmax, and ATP concentration for use in Reaction Biology's DGK screening assays are summarized in the Figure 5.



DGK isoform	DGKα	DGKβ	DGKγ	DGKδ	DGKη	DGKκ	DGKε	DGKζ	DGKι	DGKθ
Km (μM)	129	122	54	292	80	448	559	51	276	46
Vmax (μM/hr)	42.3	30.2	12.2	28.1	6.7	5.0	16.8	33.5	38.0	16.6
[ATP] in screening assay	50 μM	50 μM	50 μM	200 μM	50 μM	200 μM	200 μM	50 μM	200 μM	50 μM

Figure 5: Km app determination of DGK isoforms

To validate the established DGK panel, we screened previously reported DGK inhibitor Calphostin C across all 10 DGK isoforms. Obtained IC_{50} values are summarized in the Figure 6.



DGK Isoform	DGKα	DGKβ	DGKγ	DGKδ	DGKη	DGKκ	DGKε	DGKζ	DGKι	DGKθ
IC_{50} (M)	3.2E-06	9.0E-07	7.2E-06	1.5E-06	1.4E-06	2.1E-05	5.5E-07	2.0E-05	3.8E-07	1.4E-06

Figure 6: Calphostin C inhibitory activity across DGK isoforms

Summary

- Isoform-specific inhibitor discovery for DGK targets is important in drug discovery.
- At Reaction Biology, we have developed activity assays for all DGK isoforms using 1,2-dilauroyl-sn-glycerol (DLG) lipid as substrate.
- We have identified suitable assay buffer conditions to achieve highest DGK activity using lipid substrate dissolved in buffer.
- Based on the apparent ATP Km values which were determined for all DGK isoforms using DLG substrate, we chose the ATP concentrations for use in the assays.
- DGK assays were validated using the previously identified DGK inhibitor Calphostin to show isoform-selective inhibitor discovery applications.

RBC is ready to help customer to develop isoform selective DGK inhibitors

References

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