Evaluations of the selectivities of the diacylglycerol kinase inhibitors R59022 and R59949 among diacylglycerol kinase isozymes using a new non-radioactive assay method

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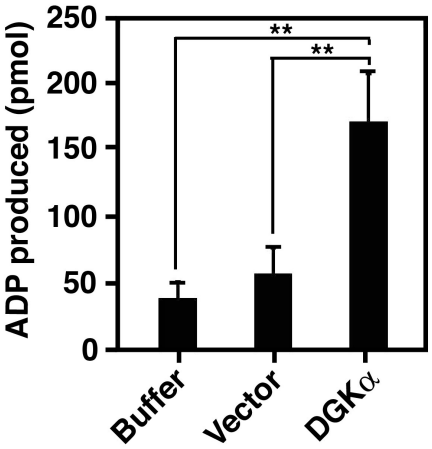
Pharmacology 2013, *in press*



	Av	SD	CV%	S/B ratio	Z'-factor
Buffer	33.0	1.0	3.0%	3.5	0.72
DGKα	117.0	6.8	5.8%		

Ten mammalian diacylglycerol kinase (DGK) isozymes (α – κ) have been identified. Recent studies have revealed that DGK isozymes play pivotal roles in a wide variety of pathophysiological functions. Thus, it is important to be able to easily check DGK activity in each patho-physiological event. Moreover, the conventional DGK assay is quite laborious because it requires the use of a radioisotope and thin-layer chromatography including multiple extraction steps. In order to minimize the laborious procedures, we established a non-radioactive, single well, two-step DGK assay system. We demonstrated that, compared to the conventional method, the new assay system has comparable sensitivity and much higher efficiency, and is effective in detecting potential agents with high reliability (Z'-factor = 0.69 ± 0.12 (n = 3)). Using the newly developed assay, we comprehensively evaluated the DGK isozyme-selectivities of commercially available DGK inhibitors, R59022 and R59949, *in vitro*. We found that, among 10 isozymes, R59022 strongly inhibited type I DGKα and moderately attenuated type III DGKε and type V DGKθ, and that R59949 strongly inhibited type I DGK α and γ, and moderately attenuated type II DGK δ and κ.

【研究室の HP】

http://pchem2.s.chiba-u.ac.jp/chem/lab/sakanelab/bfc/Top_Page.html