ProQinase
Protein Kinase SubstrateFinder

ProQinase TARGETING CANCER

Biochemical Assay Service

➢ The Service in Brief

ProQinase offers a service that allows you to get information about a substrate for your protein kinase of interest. Depending on your needs we offer to test your protein kinase with our Generic SubstrateFinder or our Physiological SubstrateFinder.

➢ ProQinase *Generic* SubstrateFinder

Identifying a suitable substrate for a given protein kinase is often a difficult task. Sometimes the physiological substrate of a protein kinase is unknown and can not be readily identified, sometimes it will not work under in vitro conditions or results in low signal strength unsuited for the requirements of high-throughput screening settings. Also some protein kinases will not accept short peptides as substrates but require proteins.

ProQinase has established more than 330 different in vitro protein kinase assays using a collection of generic serine/threonine- and tyrosine protein kinases substrates that is comprised of proteins, artificial fusion proteins, generic polypeptides and peptides.

ProQinase offers to test your protein kinase of interest against one of the following sets of generic substrates:

- **Y-Generic SubstrateFinder**: 19 generic substrates for tyrosine protein kinases
- **S/T-Generic SubstrateFinder**: 39 generic substrates for serine/theonine kinases
- **S/T/Y-Generic SubstrateFinder**: Combination of Y- and S/T-Generic SubstrateFinder for protein kinases with unknown or dual specificity

Based on our experience with 330 protein kinase these collections of generic substrate gives a very high probability to include at least one that is suitable to be used as an in vitro substrate for so far uncharacterized protein kinases. As a result of a Generic SubstrateFinder Screen you will obtain a complete report including a table of all substrates ranked according to incorporated cps, a graphical presentation of the ranking, the identity of the 3 “top scorers” , and a comparision to a maximum of two control substrates (if provided by the customer).

The screening will routinely be done with Promegas ADP-Glo™ assay technology.

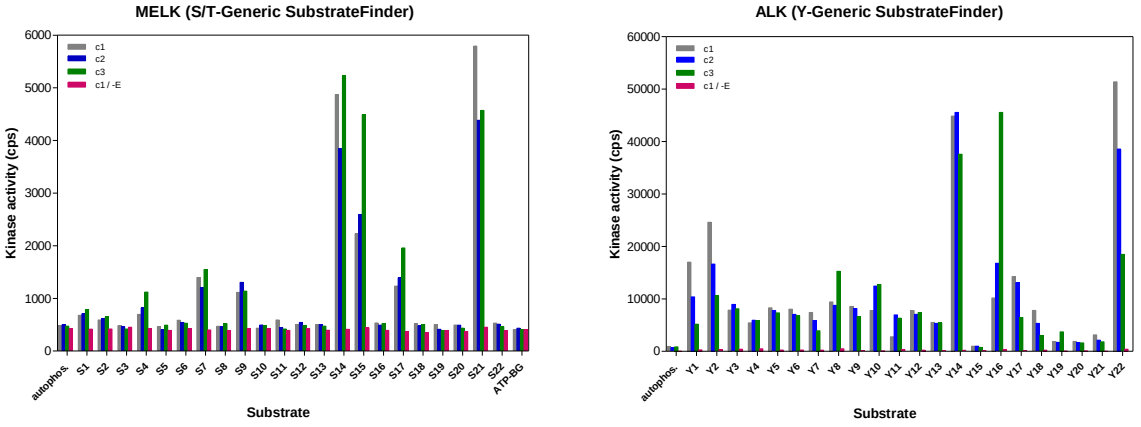

Figure 1: *Case study for Generic SubstrateFinder screens for one serine/threonine protein kinases (MELK) and one tyrosine protein kinase (ALK, anaplastic lymphoma kinase). Substrates were measured in three different concentrations (c1-c3) in singlicate at one kinase concentration. First column set represents autophosphorylation activity of the protein kinase tested. C1/-E means substrate without enzyme.*

➢ Further Service

We will verify identified substrates in customized assay setups including determination of enzymatic parameters V_{MAX} and ATP Km and/or Substrate Km. Please inquire!

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➢ ProQinase *Physiological* SubstrateFinder

ProQinase Physiological SubstrateFinder represents a service in which your protein kinase of interest is screened against libraries of biotinylated, physiological relevant peptides selected from the SwissProt and Phosphobase databases[1], using our radiometric, gold-standard ^{33}PanQinase® Assay technology.

Information you can gain from ProQinase's Physiological SubstrateFinder service include:

- Determination of protein kinase substrate consensus sequences
- Identification of potential physiological substrates/downstream targets
- Investigation of signal transduction pathways
- Identification of protein kinase peptide substrates for screening purposes

ProQinase is offering two variants of its Physiological SubstrateFinder:

- **S/T/Y-Physiological SubstrateFinder**: 720 biotinylated peptides derived from human phosphorylation sites. The S/T/Y-Physiological SubstrateFinder is suitable for any protein kinase.
- **Y-Physiological SubstrateFinder**: 145 peptides (a subset of the S/T/Y-Peptides library) with one or more tyrosine residue(s) given a total number of 370 potential tyrosine phosphorylation sites. The Y-Physiological Substrate Finder is suitable for tyrosine kinases.

As a result of a Physiological SubstrateFinder Screen you will obtain a complete report including a table of all peptides ranked according to incorporated cpm (Table 1), a graphical presentation of the ranking (Figure 2A), and a comparison of the 5 "top scorers" compared to a maximum of two control substrates (if provided by the customer) (Figure 2B).

Rank	Pos	CPM	Sequence	Pos		
1	B-B4	71808	GSPSKSPSKKKKK	B-B4	ADDB_HUMAN	2-OXOISOVALERATE DEHYDROGENASE ALPHA SUBUNIT, MI
2	A-J4	24791	AALRQLRSPRRTQ	A-J4	B343-A	RAS-RELATED PROTEIN RAB-4A
3	B-H23	23586	PINGSPRTPRRGQ	B-H23	RB_HUMAN	BAND 3 ANION TRANSPORT PROTEIN (ANION EXCHANGE PR
4	A-A16	21750	RKPGLRRSPIKKV	A-A16	MYBB_HUMAN	MYB-RELATED PROTEIN B (B-MYB).
5	A-M23	19123	LLPTPPLSPSRRS	A-M23	MYC_HUMAN	MYC PROTO-ONCOGENE PROTEIN (C-MYC).

Table 1: *Five peptides of the S/T/Y-Physiological set, showing the highest activity in a case study with kinase CDK1/CycB1. A peptide originating from RAS-related protein RAB-4A (a known CDK1 substrate[2]) is showing up at rank 2.*

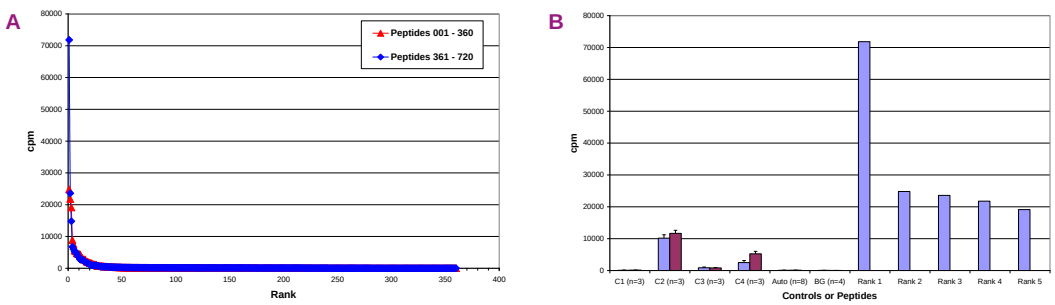

Figure 2: A*. Dynamic range of peptides of the S/T/Y-Physiological set, ranked according their degree of phosphorylation by CDK1/CycB1;* ***B****. Controls and "top scorer" in the CDK1/CycB1 assay.*

➢ Further Service

We will verify identified substrates in customized assay setups including determination of enzymatic parameters V_{MAX} and ATP Km and/or Substrate Km. Please inquire!

[1] *Peptide libraries are purchased from JPT Peptide Technologies GmbH, Berlin, Germany (www.jpt.com)* JPT

[2] *van der Sluijs P, Hull M, Huber LA, Mâle P, Goud B, Mellman I. EMBO J. 1992 Dec;11(12):4379-89*