

## NON-KINASE ENZYME ASSAYS

Most of the enzymatic assays are developed and validated for identification of competitive, reversible inhibitors. Various technologies are used such as TR-FRET, direct fluorescence and luminescence. The substrate is either the natural substrate or a surrogate substrate, like a peptide, that mimics the natural substrate.





































### PHOSPHATASES

Reversibility of protein tyrosine residue phosphorylation is an important factor in the intra-cellular signalling as phosphorylation induces conformational changes, creates docking sites for other proteins and causes intracellular relocation. This reversibility relies on the co-ordinated actions of protein tyrosine kinases and protein tyrosine phosphatases (PTPs).









































Protein tyrosine phosphatases are divided in receptor PTPs (rPTPs) and non receptor PTPs (nrPTPs), the dual specificity PTP (dsPTPs) and the low molecular PTP families. They are involved in cell-substrate adhesion, cell-cell adhesion and insulin signalling as described in Protein tyrosine phosphatase end signaling, *Stoker, A.S. (2005), J. Endocrinol., 185: 19-33.*

In this context, Cerep has designed a high throughput profiling platform to determine the inhibitory activity of compounds on the phosphatases superfamily. The robustness, reproducibility and relevance of this platform were determined by screening a panel of commercially available reference inhibitors in three independent experiments.

Cerep's panel of phosphatase assays has increased from 15 in 2009 to 24 as of January 1, 2010.

Family/assay	Ref.	Family/assay	Ref.
 phosphatase PP1 $\alpha$	 2656	phosphatase MKP1	 2652
phosphatase 1B (PTP1B)	 2593	phosphatase MKP2	 2653
phosphatase 2A (PP2A)	 2584	phosphatase MKP3	 2654
phosphatase 2B	0365	phosphatase MKP6	 2655
phosphatase CD45	 2594	 phosphatase PEST (PTPN12)	 2809
 phosphatase CDC25A	 2561	 phosphatase PTP $\beta$ (PTPRB)	 2802
 phosphatase CDC25B	 2541	 phosphatase PTP $\gamma$ (PTPRG)	 2804
 phosphatase CDC25C	 2562	 phosphatase PTP $\mu$ (PTPRM)	 2805
 phosphatase DEP1 (PTPRJ)	 2801	phosphatase SHP1 (PTPN6)	 2597
 phosphatase LAR (PTPRF)	 2803	phosphatase SHP2 (PTPN11)	 2533
 phosphatase MEG1 (PTPN4)	 2807	 phosphatase TC-PTP (PTPRN2)	 2806
 phosphatase MEG2 (PTPN9)	 2808	phosphatase VHR (DUSP3)	 2598

### PROTEASES

Family/assay	Ref.	Family/assay	Ref.
<b>SERINE PROTEASES</b>		<b>ASPARTIC PROTEASES</b>	
cathepsin G	 0179	BACE-1 ( $\beta$ -secretase)	 0701
 dipeptidyl peptidase IV (DPP-IV)	 2942	cathepsin D	 0650
elastase	 0183	cathepsin E	1001
HNE (human neutrophil elastase)	 0562	HIV-1 protease	 0346
kallikrein	 0791		
trypsin	 0512	<b>METALLOPROTEASES</b>	
<b>CYSTEINE PROTEASES</b>		ACE	 0979
caspase-1	 0680	 ACE	0302
caspase-2	 0681	ACE-2	 1954
caspase-3	 0774	ECE-1	 0184
caspase-4	 0963	 ECE-1	0313
caspase-5	 0964	MMP-1	 0510
caspase-6	 0908	MMP-2	 0489
caspase-7	 0909	MMP-3	 0490
caspase-8	 0668	MMP-7	 0502
caspase-9	 0675	MMP-8	 0643
caspase-10	 0910	MMP-9	 0491
cathepsin B	 0178	MMP-12	 0511
cathepsin H	 0801	MMP-13	 0632
cathepsin L	 0802	MT1-MMP (MMP-14)	 0670
cathepsin X	 0931	neutral endopeptidase	 0509
		TACE	 0702

 new assay  new protocol  tissue assay  binding assay  human

## PHOSPHODIESTERASES ■

Cyclic nucleotide phosphodiesterases (PDEs), ubiquitously distributed in mammalian tissues, play a major role in cell signaling by hydrolyzing cAMP and/or cGMP. Due to their diversity and specific distribution at cellular and subcellular levels, PDEs can selectively regulate various cellular functions. Increased understanding of their function at the cell and molecular level provides an impetus for the development of isoenzyme selective inhibitors for the treatment of various diseases. Examples are PDE3 inhibitors for congestive heart failure, PDE4 inhibitors for inflammatory airways disease and the most well known, inhibitor for erectile dysfunction (Viagra).

As PDEs are expressed in a variety of tissues, selectivity is a prerequisite for a therapeutically applicable PDE inhibitor. As an example, high selectivity for PDE5 inhibitors is important for treatment of erectile dysfunction to minimize the possibility of side effects that arise as a result of inhibition of other PDEs. Possible side effects include heart rate increase and vasodilation that are attributed to inhibition of PDE1 and PDE3, or blue-green vision disturbances that are attributed to inhibition of PDE6.

In this context, Cerep has designed a high throughput profiling platform to determine the inhibitory activity and selectivity of compounds on the PDE superfamily. The robustness, reproducibility and relevance of this platform was determined by screening a broad panel of commercially available reference inhibitors and known clinical drugs in three independent experiments. Poster available upon request.

For **cellular phosphodiesterase assays**, please contact us at [customresearch@cerep.com](mailto:customresearch@cerep.com)

Family/assay	Ref.	Family/assay	Ref.
PDE1B	2431	PDE5 (non-selective)	0204
PDE2A	2426	PDE6 (non-selective)	0478
PDE3A	2432	PDE7A	2351
PDE3B	2705	PDE8A	2355
PDE4A	2342	PDE10A	2357
PDE4B	2413	PDE11A	2358
PDE4D	2434	rolipram - antagonist radioligand	0379

## NO SYNTHASES ■

Family/assay	Ref.	Family/assay	Ref.
inducible NOS	0196	constitutive NOS (endothelial)	0197

## ARACHIDONIC ACID METABOLISM ■

Family/assay	Ref.	Family/assay	Ref.
<b>PHOSPHOLIPASE</b>		<b>TXA<sub>2</sub> SYNTHETASE</b>	
PLA <sub>2</sub>	3176	TXA <sub>2</sub> synthetase	0564
PLA <sub>2</sub>	2999	<b>LIPOXYGENASES</b>	
<b>CYCLOOXYGENASES</b>		5-lipoxygenase	0772
COX <sub>1</sub>	0726	12-lipoxygenase	0883
COX <sub>2</sub>	0727	15-lipoxygenase	0190

## MONOAMINE & NEUROTRANSMITTER SYNTHESIS & METABOLISM ■

Family/assay	Ref.	Family/assay	Ref.
acetylcholinesterase	0363	MAO-A - antagonist radioligand	0443
COMT (catechol-O-methyl transferase)	0457	MAO-B	0621
GABA transaminase	0461	MAO-B - antagonist radioligand	0444
HNMT (histamine N-methyl transferase)	0463	PNMT (phenylethanolamine N-methyltransferase)	0464
MAO-A	0191	tyrosine hydroxylase	0214

## SECOND MESSENGER SYSTEMS ■

Family/assay	Ref.	Family/assay	Ref.
<b>CYCLASES</b>		<b>PHOSPHOLIPASE C</b>	
adenylyl cyclase (basal)	0172	PLC	2837
adenylyl cyclase (stimulated)	0173	<b>INOSITOL PHOSPHATE</b>	
guanylyl cyclase (basal)	3004	IP <sub>3</sub> - agonist radioligand	0083
guanylyl cyclase (stimulated)	3005		

## NO SYNTHASES ■

Family/assay	Ref.	Family/assay	Ref.
ATPase (Na <sup>+</sup> /K <sup>+</sup> )	2009	ATPase (H <sup>+</sup> /K <sup>+</sup> )	0445

new assay new protocol tissue assay binding assay human

## BIOCHEMICAL ENZYME ■

Family/assay	Ref.
■ arginase 1	■ 3117

## MISCELLANEOUS ENZYMES ■

Family/assay	Ref.	Family/assay	Ref.
acetyl CoA synthetase	0388	HDAC8	■ 2247
carbonic anhydrase II	■ 2572	HDAC9	■ 2611
■ CENP-E	■ 2150	HDAC10	■ 2662
DNA polymerase I	■ 2348	HDAC11	■ 2663
DNA polymerase β	■ 2445	HMG-CoA reductase	0187
■ Eg5	■ 2151	myeloperoxidase	■ 0193
HDAC1	■ 2491	sirtuin 1	■ 2581
HDAC2	■ 2492	■ sirtuin 1 (activation)	■ 2991
HDAC3	■ 2083	sirtuin 2	■ 2582
HDAC4	■ 2493	sirtuin 3	■ 2583
HDAC5	■ 2494	topoisomerase II	■ 2430
HDAC6	■ 2495	xanthine oxidase/superoxide O <sub>2</sub> <sup>-</sup> scavenging	0276
HDAC7	■ 2610		

## ■ TESTING CONDITIONS

## ■ SUGGESTED TESTING

Primary screening at 1-10 μM in duplicate (2 wells), followed-up for IC<sub>50</sub>/Ki or EC<sub>50</sub> determination (8 concentrations in duplicate (16 wells) when compound displays more than 50% inhibition of control value or 50% stimulation relative to control.

■ SAMPLE SIZE (including IC<sub>50</sub> follow-up studies)

Assuming a molecular weight ≤ 500 g/mol and a testing concentration of 10 μM in duplicate (including a possible retest).

INDIVIDUAL CATALOG ASSAYS	SCREENING		SCREENING + FOLLOW UP <sup>1</sup>	
	WEIGHT (pre-weighed)	VOLUME (100% DMSO)	WEIGHT (pre-weighed)	VOLUME (100% DMSO)
1 to 3 assays	1 mg	25 μL@10 mM	1 mg	60 μL@10 mM
4 to 5 assays	1 mg	35 μL@10 mM	1 mg	70 μL@10 mM
6 to 10 assays	1 mg	50 μL@10 mM	1 mg	100 μL@10 mM
11 to 15 assays	1 mg	65 μL@10 mM	1 mg	110 μL@10 mM
16 to 20 assays	1 mg	75 μL@10 mM	1 mg	125 μL@10 mM
21 to 40 assays	1 mg	100 μL@10 mM	1.5 mg	250 μL@10 mM
41 to 50 assays	1 mg	150 μL@10 mM	2 mg	275 μL@10 mM
51 to 70 assays	1.5 mg	225 μL@10 mM	2.5 mg	400 μL@10 mM
71 to 100 assays	2 mg	300 μL@10 mM	3 mg	550 μL@10 mM
101 to 135 assays	2 mg	350 μL@10 mM	4 mg	650 μL@10 mM
136 to 150 assays	2.5 mg	400 μL@10 mM	4 mg	750 μL@10 mM
151 to 200 assays	3 mg	500 μL@10 mM	5 mg	1000 μL@10 mM
201 to 250 assays	3.5 mg	600 μL@10 mM	inquire	inquire

<sup>1</sup> Assuming ~10% of test in IC<sub>50</sub>. Usually, for 1 IC<sub>50</sub>: 30 μL@10 mM and + 25 μL@10 mM by additional IC<sub>50</sub>.

## ■ REQUESTED COMPOUND INFORMATION

To reduce the registration time and ensure that all the appropriate information is available to start the study in a shortest possible timeframe, please use **Cerep compound submission form** <sup>1</sup> or MS Excel file <sup>2</sup>, and provide the following **compound information**:

- ▶ **Name (compound ID) / Batch # / Molecular weight** <sup>3</sup> / **Formula weight** <sup>4</sup> / **Stock concentration** / **Stock solvent** / **Quantity** / **Unit** <sup>5</sup> / **Form** / **Storage conditions** / **Solubility**, as well as **Plate ID / plate position** for compounds delivered in plates, **Comments** <sup>6</sup>, and **Quotation number**.

**NOTE:** Impurity and colored compounds might affect the results (compound color information is mentioned in the study report).

<sup>1</sup> Cerep compound submission form will be emailed to you with your quotation. A copy can be requested from sales@cerep.com, or downloaded from Cerep website: www.cerep.com/Catalog Online

<sup>2</sup> Systematically required for studies of 10 compounds or more.

<sup>3</sup> Molecular weight (MW) of free acid or base form.

<sup>4</sup> Formula weight (FW) including salt form and/or hydrate form if applicable.

<sup>5</sup> mg<sup>g</sup>, mL<sup>g</sup>

<sup>6</sup> e.g. useful information such as sensitivity to light, stability or hygroscopicity issues.

► **General remarks:**

- If compound(s) are supplied as a stock solution in plate(s) (preferred format for any submission of 10 or more compounds), please leave columns 1 and 12 empty in a 96W plate. The 384W plate format is also acceptable with columns 1, 2, 23 and 24 empty. For any other plate format, please inquire.
- If compound(s) are not soluble in 100% DMSO, please provide any useful information concerning the solubility of the compound. The following solvents are compatible with most of our assays: DMSO (Cerep standard), H<sub>2</sub>O, Methanol, Tris/HCl 10 mM pH 7.4.
- Organic solvents such as acetone, chloroform, ether, acetonitrile, tetrahydrofuran and trifluoroacetic acid are not recommended as they will significantly affect the results from many *in vitro* assays, even at very low concentrations.

**WARNING:** Cerep will apply the standard solubilization process (described in the flow chart page 6) when compounds are received at the testing site, unless special instructions are provided with the compounds.

Customized handling procedure of compounds can be accommodated, please inquire for pricing conditions.

■ **PROTOCOL**

A typical protocol includes a minimum of 6-control wells (background, and maximal signal with and without vehicle) plus an 8-point dose-response of the relevant reference compound.

The reference compound for each assay is listed in each assay description. The historical average IC<sub>50</sub> value is also shown in each assay description.

► ANY OF OUR ASSAY PROTOCOLS CAN BE CUSTOMIZED: PLEASE INQUIRE

■ **DELIVERABLES**

Percent inhibition (mean of replicates), individual values as percent of control, EC<sub>50</sub> or IC<sub>50</sub> value (calculated from a minimum 5 concentration testing), Hill coefficient (nH), and plotted EC<sub>50</sub> or IC<sub>50</sub> curves.

■ **DATA TURNAROUND**

Complete data set is typically available within 3 weeks, after receipt of the compounds at the testing site (providing that we receive all available information to initiate the study).

Secure, password-protected data can be viewed on line as soon as they are produced, after scientific approval and QC-ed by an experienced technician.



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**ASSAY CATALOG REFERENCES**

In 2009, Cerep finalized one of the milestones in the industrialization process: the implementation of new referencing and supply chain management systems. The references of each of the assays have thus been simplified: each reference will now be displayed as 4 digits.

A correlation table between old and new assay references is available at [www.cerep.com CATALOG ONLINE](http://www.cerep.com/CATALOG ONLINE)  
<<http://www.cerep.com/Cerep/Users/pages/Catalog/Assay/catalog.asp>>

**QUESTIONS OR CONCERNS?**

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