PDEnRich™

Cyclic Nucleotide Phosphodiesterase Enrichment Reagent

- Ratio of Total Phosphodiesterase Activity* relative to total protein content increases 4-10X
- PDE protein recoverable, ~70 µg
- Up to 50X concentration of PDE volume, relative to starting volume can be obtained
- 45 minute, scaleable protocol compatible with functional assays, electrophoresis and Mass Spec

Cyclic nucleotide phosphodiesterases (PDEs) hydrolyze the secondary messengers cyclic AMP (cAMP) and cyclic GMP (cGMP) at their 3′-phosphodiester bond, to yield 5′-adenosine monophosphate (5′AMP or AMP) and 5′-guanosine monophosphate (5′GMP or GMP) respectively. These secondary messengers maintain homeostasis and thus play a pivotal role in regulating cellular pathways.

While inhibition of this class of enzymes has had clinical success, a major challenge in designing inhibitors that specifically inhibit PDE subtypes has been the sequence conservation of the catalytic domain among PDE subfamilies. Thus, new methods that can prospect into the structure and the functional properties of conformational variants of PDEs are urgently needed, as the success of PDE inhibitors will depend upon such characterization. The development of a robust enrichment method would be a crucial first step for drug discovery and biomarker classification within the PDE class. However, classical substrate affinity methods have not evolved because of the instability of the cyclic phospho-ester bond.

PDEnRich™ is a new reagent kit used for the enrichment and isolation of cyclic AMP (cAMP) and cyclic GMP (cGMP) phosphodiesterases. PDEnRich™ was discovered upon screening a silica-based, multi-dimensional surface library and optimized for the enrichment of phosphodiesterase activity*. The standard prep protocol starts with 100 µl of tissue/cell extracts, or approximately 0.5 mg total protein, but the process can be scaled up or down to accommodate different sample volumes and protein concentrations. The kit includes all necessary reagents for immediate use

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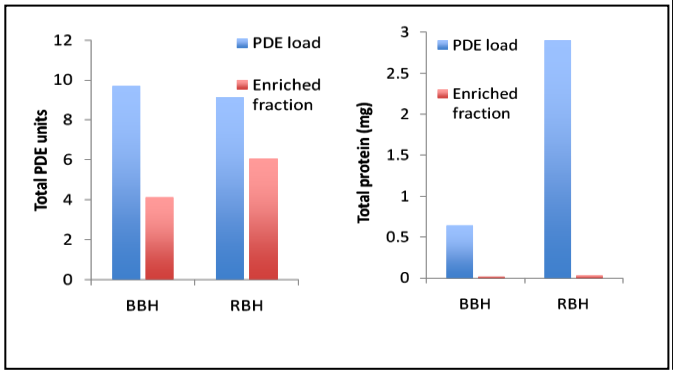

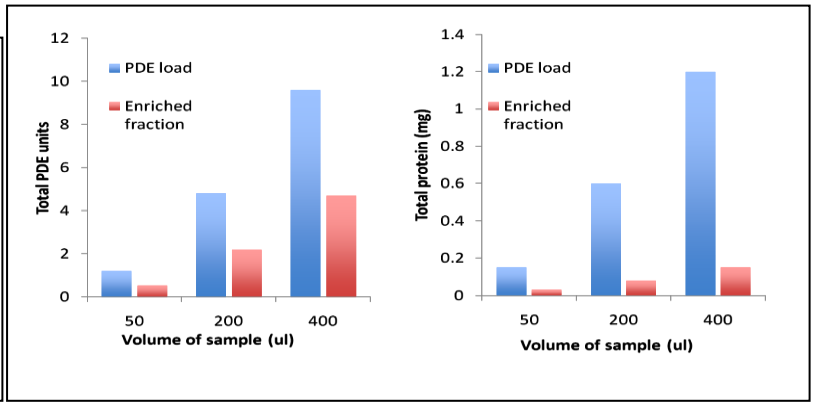

On the left is a comparison of enriched Bovine Brain Homogenate (BBH) and Rat Brain Homogenate (RBH) before and after treatment with PDEnRich™. A substantial amount of cAMP hydrolysis activity* is recovered in both cases with a dramatic reduction in total protein content. On the right, similar results are achieved with different amounts of protein volumes applied. *As measured by a previously published UV/visible spectrophotometry-based kinetic assay which quantifies the oxidation of NADH, coupled to formation of 5′AMP using three coupling reactions catalyzed by adenylate kinase, pyruvate kinase and lactate dehydrogenase (Chock and Huang, 1984).

Product	# of preps	Item No.
PDEnRich™ 10	10	PD545-10
PDEnRich™ 50	50	PD545-50

Kit Contains:	**PDEnRich™ 10**	**PDEnRich ™ 50**
PDEnRich™ reagent powder	0.5 g	2.5 g
Cleanascite™***PRO*** reagent suspension	1 ml	5 ml
PD-BB Binding Buffer	8 ml	40 ml
PD-EB Elution Buffer	2 ml	10 ml
Spin-filters	10	50

Storage: 3-5 days at Room Temperature, For long-term, 4°C.

Sample Preparation: Each prep processes approximately 1- 2 mg total soluble protein, based on 100 µl of tissue homogenates with a soluble protein content in the 10 – 15 mg/ml range. Larger volumes of lower protein content can also be used, but sample preparation volumes should be proportioned accordingly. Delipidation is recommended for most samples. **Cleanascite**™***PRO*** supplied as part of the kit, is a useful general clarification and delipidation reagent. Lipid biomass can vary greatly, so the ratios shown are only intended to provide general guidance in use. The ideal pH for samples should be around 7.

1. Resuspend **Cleanascite**™ ***PRO*** by gentle shaking.
2. Add 1 volume of **Cleanascite**™ ***PRO*** to 5 volumes of the sample. Mix the sample for 10 minutes.
3. Centrifuge sample at 16,000 G's (maximum microcentrifuge).

The supernatant should be clarified and free from colloidal biomass. Pipette off "delipidated sample".

- Dilute above supernatant in the ratio of 1 volume of sample + 1 volume of **PD-BB** Binding Buffer. Use this "prepared" sample for PDE enrichment in step 3 below.

Surface Preparation. 50mg of **PDEnRich**™ **surface reagent** powder per prep should be used. The powder should be added to the filter cup of the Spin-X tube provided as a part of the kit. Then,

1) Add 200 ul of **PD-BB binding buffer** to the **reagent powder** and mix for 3 minutes. Centrifuge and discard the flow-through.
2) Repeat step one, once more.

PDE enrichment.

3) Add 200 µl of "prepared" sample (diluted in Binding Buffer as given in sample preparation) to the surface pellet obtained at the end of step 2 above. Mix until pellet is homogeneously resuspended, and shake the mixture for 10 minutes. Centrifuge and discard the flow-through.
4) Add 200 µl of **PD-BB** Binding Buffer to surface pellet. Mix until pellet is homogeneously resuspended, and then shake it for 3 minutes. Centrifuge and discard the flow-through.
5) Repeat step four once more.
6) Add 200 µl of **PD-EB** elution buffer to the surface pellet. Mix to homogeneously resuspended the pellet. Shake the sample for 10 minutes. Centrifuge and collect the flow-through. **This is the PDE enriched fraction**.

For optimal results, the volumes may need to be adjusted up or down to account for differences in specific activity and other sample matrix factors. The elution buffer is pH 9, so the activity measurements must compensate for either higher pH, dilutions to neutrality, or buffer exchange.

Contact Us

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PDEnRich™ was developed jointly by ProFACT Proteomics Inc., and Biotech Support Group LLC. It can be purchased through them or their authorized distributers.