

Certificate of Analysis

BioForm (100 assays)

Catalog # 12-711

Lot # PAT_A0001

Description: BioForm, developed by P.A. Technologies, LLC located in Amherst MA, is a lipid-based nanoparticle with nickel-chelating moieties for use in assembling purified histidine-tagged proteins which are normally membrane associated. BioForm allows the end user to study membrane-associated proteins in a way that restores the essential biological environment created by the cell membrane. This synthetic template restores the organizing features of the membrane without the difficulties of membranous preparations and reconstitution procedures.

Formulation: 30 μ L of 1.0 nM. BioForm is compatible with TCEP, moderate amounts of β -mercaptoethanol (BME) (< 1mM w/v), mild detergents (< 0.01% - see FAQ), and will tolerate EDTA and EGTA to 10 μ M. BioForm has been used successfully with Tris, MOPS, and HEPES buffers from pH 6.5 to 7.5. BioForm is shipped in 50 mM Tris buffer, pH 7.0.

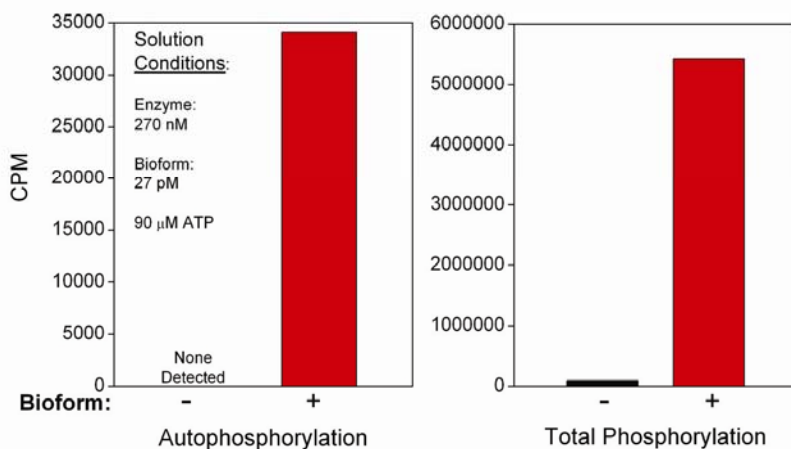
Storage and Stability: Stable for 90 days at 2-8 $^{\circ}$ C. **Do Not Freeze.**

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

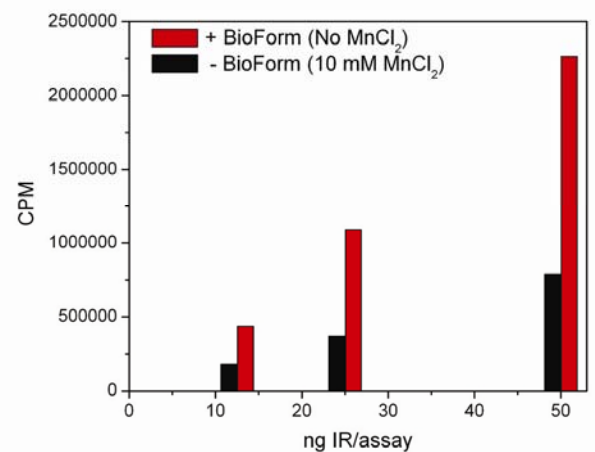
Quality Control Testing – Millipore’s Insulin Receptor (14-466), and IGF-IR (14-465)

Insulin Receptor Phosphorylation

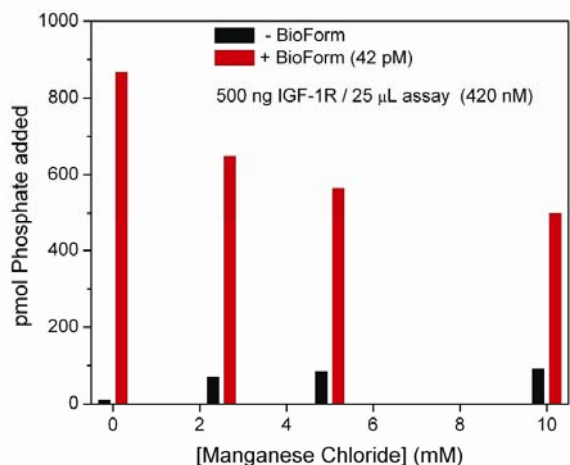
(No Manganese Chloride)



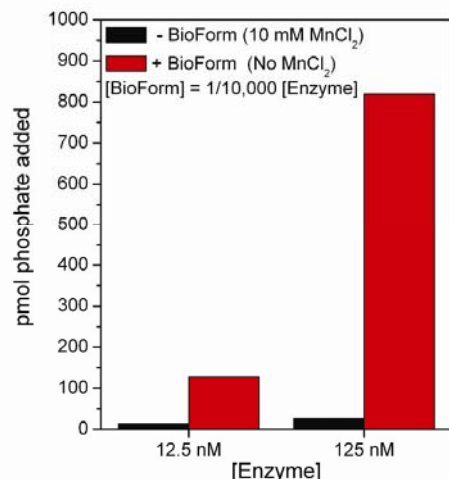
Insulin Receptor Phosphorylation of AxlTide



IGF-1R Phosphorylation of IGF-1Rtide



IGF-1R Phosphorylation of IGF-1Rtide



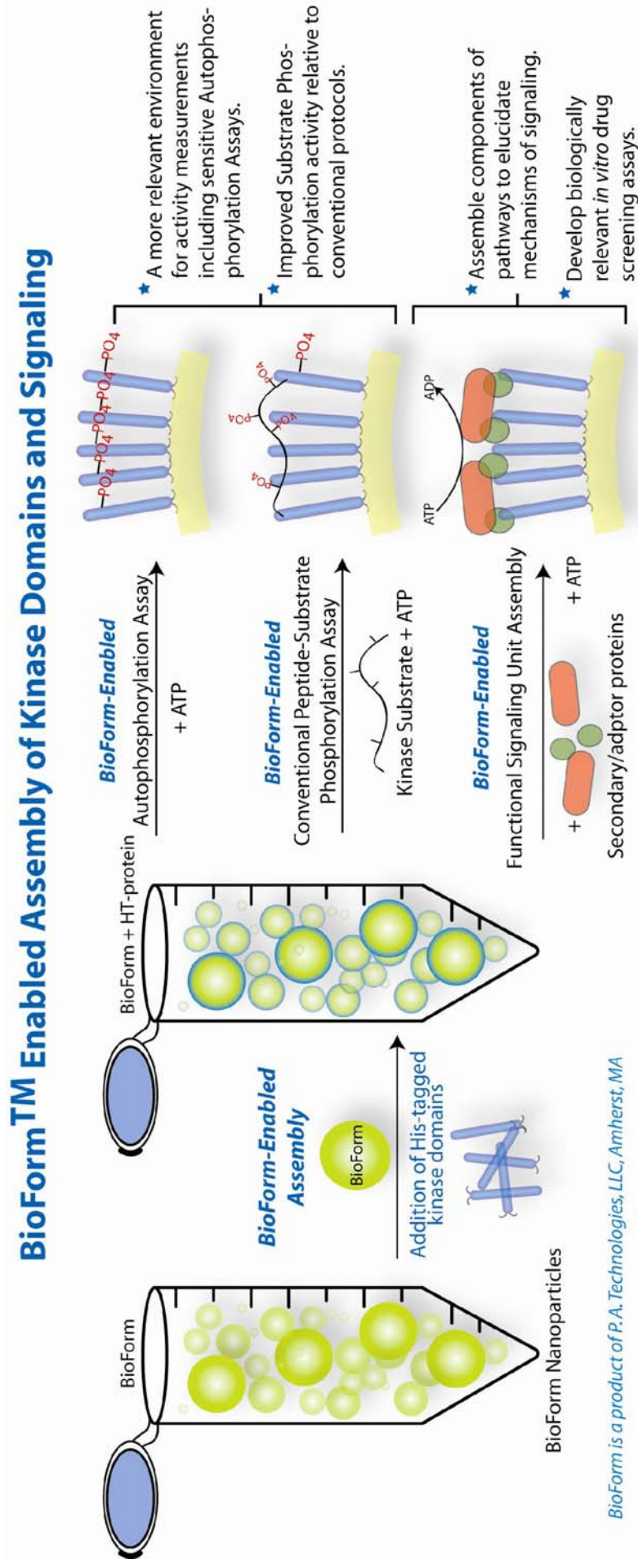
Partial list of *BioForm*
Compatible Kinases available
from Millipore

BTK	14-552
EphB2	14-553
IGF-1R	14-465
IR	14-466
Met	14-526
Tie2	14-540
TrkA	14-571
Axl	14-512
ErbB4	14-569
Lck	14-442
Fak	14-720
PKC γ	14-486
Flt1	14-562

Assay Protocol

- 1) His-tagged proteins, including kinases or other membrane associated proteins may be templated using BioForm, a P. A. Technologies product. BioForm is added at 1/10000 (one ten-thousandth) molar concentration of the his-tagged protein of interest. For example, BioForm is provided at 1.0 nM, if an experiment calls for 100 nM protein, BioForm is added at 10 pM. It is recommended optimizing the concentration of BioForm depending on your experimental plan as banding capacity and strength will change with each protein, e.g. 5 pM to 20 pM BioForm. (Each BioForm particle has the capacity to bind multiple protein fragments.)
- 2) Follow standard assay protocol for your protein. In some cases, template-directed assembly will eliminate the necessity of adding excess metals to the background buffer, e.g. manganese, and other components as well; simplifying the buffer requirements. We recommend following established protocols for your protein and optimizing the assay as needed (*i.e.* perform assay +/- manganese or at lower [Mn²⁺]).
- 3) Adaptor and secondary proteins may then be added (these should not be his-tagged unless your intent is to similarly localize them at the surface of the template – see FAQs at www.millipore.com) for analysis of pathway branches (see Figure 1 below).

Figure 1. Scheme of how BioForm transforms conventional kinase assays.



BioForm is a product of P.A. Technologies, LLC, Amherst, MA