

ProFluor™ Ser/Thr Phosphatase Assay

Description

The ProFluor Ser/Thr Phosphatase Assay^(a,b) measures the activity of purified serine/threonine protein phosphatases in a multiwell plate format. The assay allows detection of protein phosphatase 1 (PP1), PP2A, PP2B, and PP2C in a simple “add, mix and read” format. The assay begins with a standard phosphatase reaction using the provided phosphorylated bisamide rhodamine 110 peptide substrate (S/T PPase R110 Substrate) and Control AMC Substrate (Figure 1). In this configuration, both the S/T PPase R110 Substrate and Control AMC Substrate are nonfluorescent. Following the phosphatase reaction, a prepared protease solution is added that simultaneously stops the phosphatase reaction and digests the dephosphorylated S/T PPase R110 and the Control AMC Substrate, producing highly fluorescent rhodamine 110 and AMC. Phosphorylated S/T PPase R110 Substrate is resistant to protease digestion and remains nonfluorescent. Thus, the R110 fluorescence intensity is correlated with phosphatase activity in the presence of active protease, and the AMC fluorescence intensity is an indication of protease activity.

A compound that only inhibits the phosphatase will decrease the R110 fluorescent signal but not the AMC signal (Figure 2). A protease inhibitor will decrease both AMC and R110 signals. To distinguish protease inhibitors from phosphatase inhibitors, the AMC fluorescence signal should be examined carefully where a decrease in R110 fluorescence is observed.

Benefits:

Homogeneous and Non-radioactive: Simple “add, mix and read” format with none of the safety and disposal costs associated with radiometric assays.

Sensitive: Requires just nanogram quantities of enzyme per well.

Highly Predictive Results: Signal-to-background ratios of 20:1 and low well-to-well variability result in Z' values of 0.8 or greater, which ensures in highly predictive results (Figure 3).

Have more confidence in your results: The micromolar concentration of substrate used produces fluorescent signals much higher than the intrinsic fluorescence of most problematic test compounds.

Low False-Positive Rate: The Protease Reagent in the ProFluor Ser/Thr PPase Assay was tested against the Library of Pharmacologically Active Compounds (LOPAC-Sigma-RBI). None of the 640 LOPAC library compounds interfered with the Protease Reagent (data not shown). A control peptide (AAF-AMC) is included that can monitor protease activity (Figure 2).

Accurate IC₅₀ Values: IC₅₀ values obtained using the ProFluor Ser/Thr PPase Assay for all measured PPase inhibitors agree closely with published values (Figure 4).

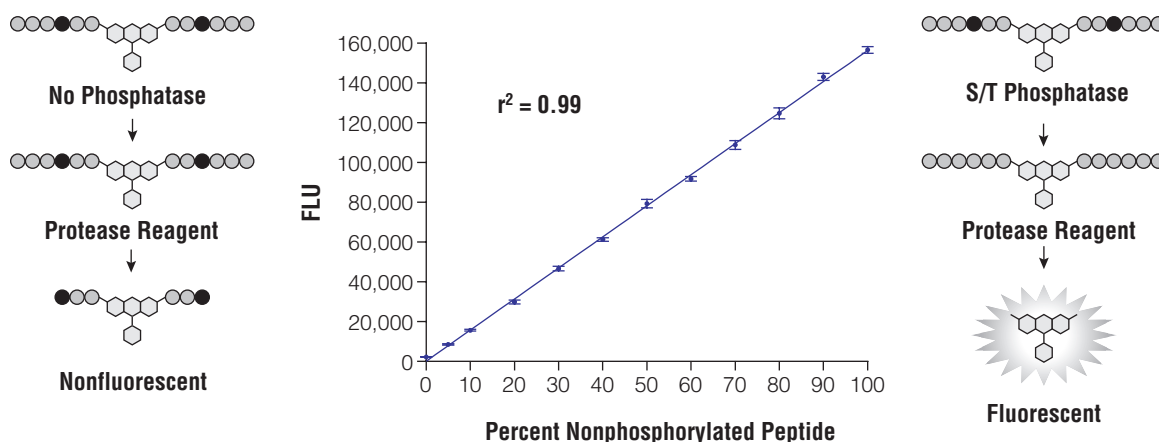


Figure 1. Effect of phosphopeptide content on fluorescence intensity. The graph shows the average FLU (n = 8) obtained after a 90-minute Protease Reagent digestion using mixtures of nonphosphorylated S/T PPase R110 substrate and phosphorylated S/T PPase RT110 substrate as indicated to mimic a phosphatase titration. (FLU = Fluorescence Light Units, excitation wavelength 485nm, emission wavelength 530nm; $r^2 = 0.999$). Phosphorylated amino acids (Dark circles in the illustration) block removal of amino acids by the protease.

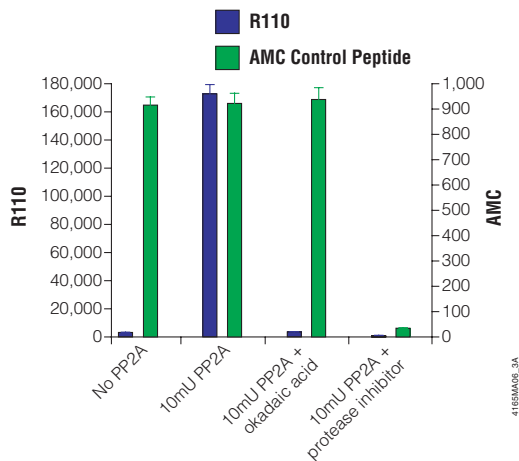


Figure 2. Control AMC Substrate reduces false positive hits. Dark bars indicate fluorescence using excitation at 485nm and emission at 530nm (S/T PPase R110 Substrate). Gray bars indicate fluorescence using excitation at 355nm and emission at 460nm (Control AMC Substrate). The assay was performed in solid black, flat-bottom 96-well plates using the conditions indicated. The results demonstrate that a compound that only inhibits the phosphatase will produce a decrease in R110 fluorescence but not AMC fluorescence, while a protease inhibitor will decrease both fluorescence signals.

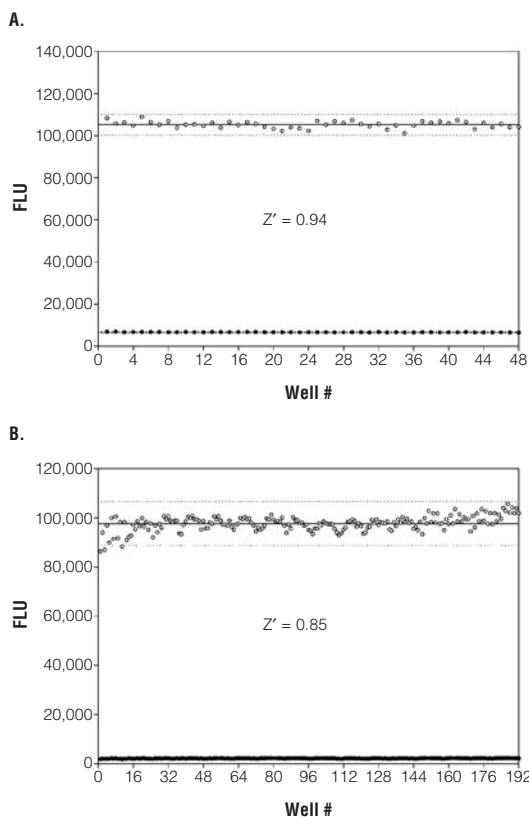


Figure 3. Z'-factor values of greater than 0.8 are routinely obtained in either 96- or 384-well formats. The assay was performed in solid black, flat bottomed plates with (open circles) and without (solid circles) phosphatase. FLU = Fluorescence Light Units. **Panel A:** 200ng/well PP2B was used. **Panel B:** 6.25 milliunits/well PP1 was used as described in the ProFluor™ Ser/Thr Phosphatase Assay Technical Bulletin (#TB324).

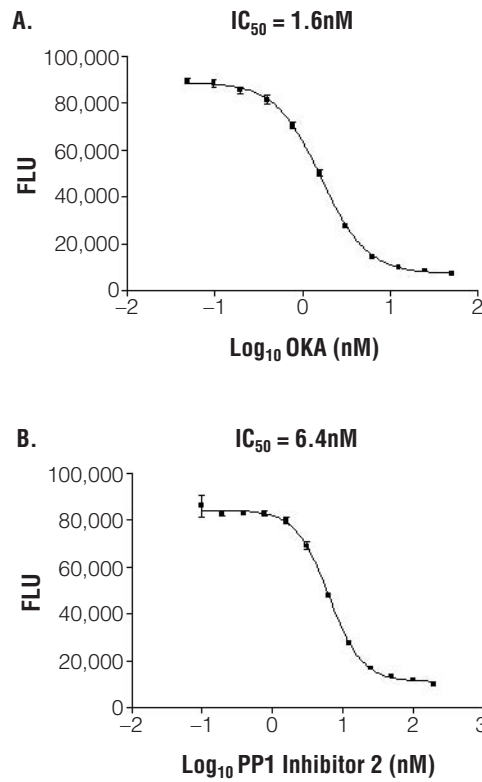


Figure 4. Accurate IC₅₀ values. Published literature IC₅₀ value for okadaic acid (OKA) for PP2A (Panel A) is approximately 0.6nM, while the published literature value for PP1 Inhibitor 2 (Panel B) is around 3.0nM. FLU = Fluorescence Light Units.

Ordering Information

Product	Size	Cat. #
ProFluor™ Ser/Thr PPase Assay ^(a,b)	4 plate	V1260
	8 plate	V1261

Component listing for V1260:

- 12µl S/T PPase R110 Substrate, 10mM
- 12µl Control AMC Substrate, 10mM
- 240µl Protease Reagent
- 1ml MgCl₂, 1M
- 1ml MnCl₂, 20mM
- 1ml CaCl₂, 100mM
- 1ml NiCl₂, 100mM
- 12µl Stabilizer Reagent
- 6ml 5X Reaction Buffer B
- 5ml 5X Termination Buffer B

Component listing for V1261:

- 24µl S/T PPase R110 Substrate, 10mM
- 24µl Control AMC Substrate, 10mM
- 480µl Protease Reagent
- 1ml MgCl₂, 1M
- 1ml MnCl₂, 20mM
- 1ml CaCl₂, 100mM
- 1ml NiCl₂, 100mM
- 24µl Stabilizer Reagent
- 12ml 5X Reaction Buffer B
- 10ml 5X Termination Buffer B

^(a) Patent Pending.

^(b) This product is covered by U.S. Pat. Nos. 4,557,862 and 4,640,893 and is sold for research use only. All other uses, including but not limited to use as a clinical diagnostic or therapeutic, require a separate license. Please contact Promega Corporation for details relating to obtaining a license for such other use.

ProFluor is a trademark of Promega Corporation.



Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601

© 2003 Promega Corporation. All Rights Reserved.
All prices and specifications are subject to change without prior notice.

Printed in USA 09/03
11019-DS-CR
Part# DS214

