*Revised: 18–March–2001*

# *Phosphoinositol-Specific Phospholipase C Substrates*

**P-3764 1-pyrenebutyl *myo*-inositol-1-phosphate, lithium salt**
**B-7706 BODIPY® FL $C_5$ *myo*-inositol-1-phosphate, lithium salt**

## Quick Facts

***Storage upon receipt:***

- -20°C
- Desiccate
- Protect from light

## *Introduction*

Phosphoinositol-specific phospholipase C (PI-PLC) occurs in both bacteria and mammalian cells.[1,2] In eukaryotic cells, it plays an important role in signal transduction, hydrolyzing phosphoinositol-4,5-bisphosphate ($PIP_2$) to generate two second messenger species, diacylglycerol and inositol-1,4,5-trisphosphate ($IP_3$). To provide an alternative to radioisotope-based assay methods,[3] a fluorescent substrate for PI-PLC has been developed by Dr. Stewart Hendrickson and colleagues — 1-pyrenebutyl *myo*-inositol-1-phosphate (P-3764; also named 4-(1-pyreno) butylphosphoryl-1-*myo*-inositol).[4,5] The BODIPY® FL–labeled substrate (B-7706) can be excited and detected at longer wavelengths and is suitable for use with argon-ion laser–excitation sources.

Since the fluorescence of these substrates is not appreciably modified upon enzymatic cleavage, quantitation of enzyme activity requires chromatographic separation of the substrate and product. Assay procedures using these substrates for fluorescence-detected HPLC or TLC separation of PI-PLC hydrolysis products have been provided by Dr. Hendrickson. Assays of bacterial PI-PLC (from *B. cereus*) using these methods have been described.[4,5]

## *Storage*

The products are provided as solid lithium salts in 500 µg (P-3764) or 100 µg (B-7706) units. Upon receipt, these products should be stored frozen at -20ºC, desiccated and protected from light.

## *Experimental Protocol*

### *Reagent Preparation*

**Stock solution:** Prepare a 1.25 mM substrate stock solution by dissolving one 500 µg vial of P-3764 (molecular weight 522.42) in 765 µL of methanol or one 100 µg vial of B-7706 (molecular weight 554.22) in 145 µL of methanol. Store the stock solution frozen at -20ºC, protected from light.

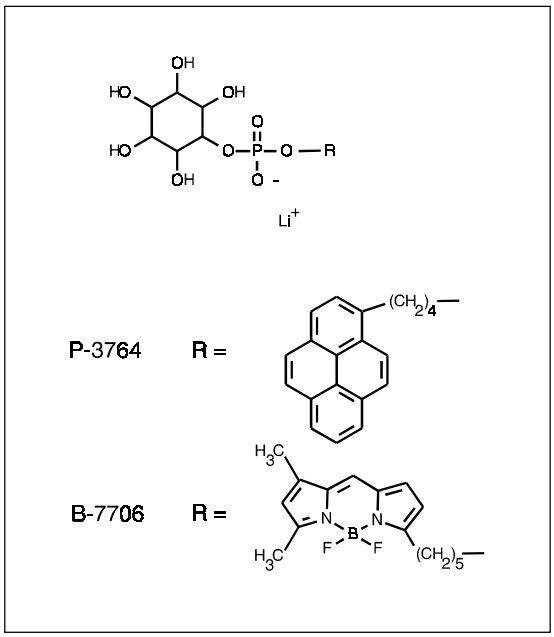


***Figure 1.*** *Structures of 1-pyrenebutyl myo-inositol-1-phosphate (P-3764) and BODIPY FL $C_5$ myo-inositol-1-phosphate (B-7706).*

**Assay solution:** For each assay, evaporate 20 µL of substrate stock solution (see *Stock Solution*) to dryness in a small tube using an argon or nitrogen stream or vacuum. Prepare the assay solution by adding 20 µL of 50 mM MES buffer (pH 7.0), using a bath sonicator to completely disperse the substrate.

**HPLC solvent:** 5 mM tetrabutylammonium dihydrogen phosphate in acetonitrile/methanol/water (70:10:20 v/v).

### *Assay*

**1.1** At time zero, mix 20 µL of the assay solution (prepared in *Reagent Preparation*) with 5 µL PI-PLC (0.2 to 4 ng of pure enzyme in 0.1% bovine serum albumin).

**1.2** Remove 5 µL aliquots of the assay mixture at various time intervals and add to 95 µL of HPLC solvent (prepared in *Reagent Preparation*) in a microfuge tube. Vortex immediately, record the elapsed time and centrifuge for 2 minutes at about 14,000 rpm.

### HPLC Analysis

**2.1** Column specifications: Reverse-phase 5 µm $C_{18}$ ODS, 25 cm × 4.6 mm, with a flow rate 1.0 mL/min.

**2.2** Fluorescence detector: For P-3764, set excitation and emission wavelengths at 343 nm and >370 nm, respectively. For B-7706, set the excitation and emission wavelengths at 485 nm and >500 nm, respectively (excitation at 228 nm and emission >470 nm is also suitable).

**2.3** Inject 20 µL of diluted sample (prepared in *Reagent Preparation*). Expected retention times for the substrate and hydrolysis product are about 2.4 minutes and 6.2 minutes respectively for P-3764 under these conditions. For B-7706, the expected retention times are 2.3 minutes (substrate) and 3.8 minutes (product).

**2.4** Calculate the degree of product release from the chromatogram peak areas for the substrate and product ($A_S$ and $A_p$):

$$\text{Product (nmol)} = \frac{(A_P)(25\ \text{nmol})}{(A_P) + (A_S)}$$

### TLC Analysis

Spot about 5 µL of the undiluted assay mixture (prepared in *Reagent Preparation*) onto a silica gel TLC plate, dry and develop with chloroform/methanol/water 65:35:3 (v/v). Detect the substrate and hydrolysis product by fluorescence using a suitable ultraviolet-excitation handlamp.

## References

**1.** Science 244, 546 (1989); **2.** Methods Enzymol 197, 493 (1991); **3.** Methods Enzymol 197, 502 (1991); **4.** Bioorg Med Chem Lett 11, 619 (1991); **5.** Biochemistry 31, 12169 (1992).

## Product Summary

*Current prices may be obtained from our Web site or from our Customer Service Department.*

| Cat # | Product Name | Unit Size |
|---|---|---|
| B-7706 | BODIPY® FL $C_5$ *myo*-inositol 1-phosphate, lithium salt | 100 µg |
| P-244 | 1-pyrenebutanol | 100 m |
| P-3764 | 1-pyrenebutyl *myo*-inositol 1-phosphate, lithium salt | 500 µg |

## *Contact Information*

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information


**Molecular Probes, Inc.**
PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

**Customer Service**: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

**Toll-Free Ordering for USA and Canada:**
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

**Technical Assistance**: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

**Molecular Probes Europe BV**
PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

**Customer Service**: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

**Technical Assistance**: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl


*Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.*