

EnzChek[®] Polarization Assay Kit for Proteases (E-6658)

Quick Facts

Storage upon receipt:

- 4°C
- Do not freeze
- Desiccate
- Protect from light

Ex/Em: 505/513 nm

Introduction

Fluorescence polarization assays have proven to be important in large-scale screening for protease activities and for inhibitors of specific proteases. Molecular Probes' EnzChek[®] Polarization Assay Kit for Proteases provides sensitive and rapid assays that are suitable for detecting the activity of metallo-, serine, acid and thiol proteases.¹⁻³ The EnzChek Kit contains a BODIPY[®] FL conjugate of casein. To maximize the sensitivity of the assay, the degree of labeling of the protein substrate has been carefully optimized for fluorescence polarization.

When a fluorescent molecule tethered to a protein is excited by polarized light, the polarization of fluorescence emission is dependent on the rate of molecular tumbling. Upon proteolytic cleavage of the fluorescently labeled protein, the resulting smaller peptides tumble faster, and the emitted light is depolarized relative to that measured with the intact protein. This technology is more sensitive than many nonradioactive protease assays and allows measurements to be taken in real time, permitting the collection of kinetic data.^{1,4} Note that some applications of this kit in fluorescence polarization may be covered by U.S. Patent 5,804,395. Purchase of the kit does not include a license under this patent.

Molecular Probes' BODIPY FL casein substrate is superior to the fluorescein thiocarbonyl (FTC)-casein substrate that has been widely used for fluorescence polarization assays. The fluorescence of the BODIPY FL dye is insensitive to pH from pH 3 to 10, permitting convenient analysis of a wide variety of proteases.

Materials

Kit Contents

- **BODIPY FL casein** (Component A), two vials that each contain 100 µg of lyophilized substrate

- **5X digestion buffer** (Component B), two bottles that each contain 25 mL of 50 mM sodium phosphate, 750 mM NaCl, pH 7.4 (5X PBS, pH 7.4) and 2 mM sodium azide

When a substrate working concentration of 1 µg/mL is used, the kit provides sufficient reagents for 100 assays with 2 mL volumes or 1000 assays with 200 µL volumes.

Storage and Handling

Upon receipt, the kit should be stored at 4°C protected from light. Allow reagents to warm to room temperature before opening vials. When stored properly, these reagents are stable for six months to one year.

The reconstituted BODIPY FL casein stock solution (see step 1.2, below) may be stored protected from light at 4°C for 2 to 4 weeks. When storing this solution at 4°C longer than 1 week, add 2 mM sodium azide. If longer storage is required, divide into aliquots and freeze at -20°C, protected from light.

Materials Required but Not Provided

- An appropriate enzyme standard of known specific activity, if applicable
- Specific buffers for detection of enzymes requiring activation compounds or a unique pH environment, if applicable (see note in step 1.1)

Experimental Protocol

Reagent Preparation

1.1 Prepare 1X digestion buffer by diluting desired amount of the 5X digestion buffer fivefold with dH₂O. **Note:** The digestion buffer provided (pH 7.4) is recommended for detecting the protease activity of most proteases active in the pH range of 7.2 to 7.6. However, if you are working with an enzyme that requires activation compounds or a unique pH environment, prepare the specific buffer required in place of the digestion buffer provided.

1.2 Prepare a 1.0 mg/mL stock solution of the BODIPY FL casein substrate by adding 100 µL of 1X digestion buffer (or an appropriate substitute buffer) directly to the vial containing the lyophilized substrate. The substrate may take several minutes to fully dissolve at room temperature.

Sample Analysis

The actual application of the EnzChek Polarization Assay Kit depends on the particular experiment and on the instrument to be used. A final concentration of 1.0 µg/mL of the BODIPY FL casein substrate is suitable for most fluorescence polarization assays. The BODIPY FL casein substrate and peptides derived therefrom have excitation/emission maxima of 505/513 nm. The

following example is a general case and assumes that a 2.0 mL assay volume is required for the instrument.

2.1 Prior to the addition of protease-containing solutions, a zero-time reading is taken for the BODIPY FL casein substrate in digestion buffer. Actual reagent volumes will depend on the fluorescence polarization instrument used. For instruments that require 2 mL volumes, add 2.0 µL of 1.0 mg/mL BODIPY FL casein stock solution to 2.0 mL of 1X digestion buffer prepared in step 1.1 (or specific buffer required) to yield a final substrate concentration of 1.0 µg/mL. Then record the zero-time fluorescence polarization reading.

2.2 Add 20 µL of protease-containing solution and gently mix reagents.

2.3 Record fluorescence polarization every 10 to 30 seconds. It may be necessary to generate serial dilutions of the enzyme solution in order to determine the appropriate range of enzyme response, optimize the linear range of the assay and, for quantitative analysis, achieve steady-state kinetics for the rate of fluorescence polarization decrease with respect to time.

2.4 For quantitating the activity of purified protease preparations by fluorescence polarization, generate several protease activity curves at several enzyme dilutions. If possible, use an appropriate enzyme standard of known specific activity that closely matches the protease activity being determined. Slopes obtained from the initial, linear portion of fluorescence polarization curves and plotted against enzyme concentration may be used to generate a standard curve.

References

1. J Biomol Screening 1, 33 (1996); 2. Anal Biochem 243, 1 (1996); 3. Arch Oral Biol 45, 1101 (2000); 4. Biotechniques 17, 585 (1994).

Product List

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

Cat #	Product Name	Unit Size
E-6658	EnzChek® Polarization Assay Kit for Proteases *green fluorescence* *100-1000 assays*	1 kit

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

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