

1. PRODUCT DESCRIPTION

GENIUL_4900017000

High Pure Propidium Monoazide

What this product does

This product is designed for photo labelling of nucleic acids present in an aqueous suspension. The nature of the reagent precludes passing through cell membranes. For this reason the DNA/RNA from cells with undamaged membrane will be free of labelling. After the treatment of microbial aqueous suspension with this product, only DNA/RNA from living or infectious microorganism will be detected by different analytical procedures such as PCR, flow cytometry or fluorescence microscopy.

Purity: >95%

Mwt: 511,49

Storage / Stability

Upon receipt, store in a lab fridge. Protect from direct light.

Contents

2 vials of 0.5 mg.

Additional Equipment Required

Photo-activation system, PhAST BLUE (Cat.No. 9000700)

Isothermal system for micro-tubes incubation, Dark Box system (Cat.No. 90001200)

Reaction tubes (Cat.No. 4900019000)

Decontamination system, D-Bag system (Cat.No. 900099645)

Applicability statement

The amount of reagent contained in standard reactions (e.g. 25 μ M) is sufficient to neutralize the DNA of at least $1 \cdot 10^5$ cells with damaged membranes. Other compounds present in the sample can interfere in different ways in the yield of the reaction. For this reason, if possible, one or more washing steps (e.g. by centrifugation-resuspension with appropriate buffer) are advisable before the use of the reagent tube.

This product has been formulated to meet the range of Propidium monoazide concentration used in public scientific works.

Centrifuge the 0.5 mg micro container to minimize the risk of reagent loss after opening.

Each vial contains 0.5 mg of High Pure Propidium monoazide that must be dissolved in a 20% DMSO solution, or sterile water, to a desired stock concentration.

In order to ensure a long conservation time, it is advisable to aliquot the stock solution in several micro tubes (during this step protect the reagent from light). Store the aliquots in the dark at -20°C and minimize the number of freeze/thaw cycles

Example:

- . Solution stock preparation

If 782 µl are added to a 0.5 mg micro container, the final concentration of the stock solution will be 1250 µM.

- . Reaction

If 10 µl of the stock solution (1250 µM) are added to 490 µl of sample, the final concentration in the reaction will be 25 µM.

78* assays can be performed with 0.5 mg micro container by using 25 µM concentration and a final reaction volume of 500 µl.

* Calculations of the number of assays will vary with the final concentration used and/or final volumes reaction.

General Rules

Please, carefully read the MSDS for this product. Follow the general procedures of a molecular biology laboratory, especially those aimed at preventing cross-contamination. It's advisable to use micro-pipette tips with filter, the use of gloves and personal protection. The use of biological safety cabinets is recommended if you believe that your sample handling system can generate aerosols.

2. OPERATING PROCEDURE

Step	Action
1	Homogenize the sample with the aid of a vortex or a micro-pipette.
2	Add propidium monoazide stock solution in the right concentration to obtain the desired final concentration in the reaction. Protect from light.
3	Homogenize the sample with the aid of a vortex or a micro-pipette.
4	Incubate in the dark at 2-8°C during at least 5 minutes <i>The incubation time, is related to sample and microorganism type. During this step if you don't have an isothermal mixer, complete the procedure by the means of a vortex.</i>
5	Proceed with the photo-activation step with a sterile transparent micro-tube

After step 5 the sample is ready for nucleic acid extraction-purification by using routine procedures. The sample is also ready for the following steps of your fluorescence microscopy method. If you use flow cytometry detection methods probably you will need an additional sample-washing step.

Most of commercial kits start the DNA extraction with sample volumes of 200 μ l; if you need to work with the entire sample volume an additional step of centrifugation and supernatant elimination must be performed.

Handle liquids containing Phenanthridium derivatives with care and follow the MSDS, for this purpose we recommend the use of D-Bag system (Cat.No. 900099645)

3. FREQUENTLY ASKED QUESTIONS

On our web page (www.geniul.com) you will find a more extensive list of FAQ's.

What is the best system for reagents photo activation?

Devices based on high power halogen lamps, have been successfully used despite of some serious limitation, e.g. overheating risk and non-homogeneous light dose.

Nowadays the PhAST blue system from GeniUL offers a better control in light dose without sample overheating. By the means of its specific software it's possible to have a total control of the photo activation process.

What is the optimum incubation time?

The incubation of the sample with the reagent on darkness conditions may be critical for some applications. It is necessary to allow the reagent to entry in all the damaged membrane cells, including spores and protozoan cysts. For most purposes, an incubation step of 5 to 15 minutes may be satisfactory. In some case longer incubation time is needed, for example, 45 minutes for protozoan cyst.

Is incubation temperature important?

Yes, incubation temperature is important for several reasons:

1) Cellular membrane fluidity is strongly influenced by temperature. The active ingredients of this product are quite soluble in hydrophobic solvents, so they have some ability to interact with cell membranes. At lower temperature, cell membranes are less fluid, so its specific interaction with the reagent and the subsequent diffusion to cytoplasm is greatly reduced.

2) Constant temperature incubation minimizes results variability.

If the treatment is not 100% effective, will I have false positives by PCR?

A treatment with a 99.9% effectiveness in a sample with $1 \cdot 10^5$ dead cells, may still contain the DNA from 100 dead cells. Therefore for some techniques with high sensitivity such as nested PCR or real-time PCR, false positives results can be obtained

For this reason, some authors recommend a result analysis based on relative rather than absolute values. (<http://www.ncbi.nlm.nih.gov/pubmed/20632000>)

Recent works suggested strong relation between signal reduction in PCR and amplicon size.

4. WARRANTY AND DISCLAIMER OF LIABILITY

GeniUL warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

- (1) The product is used according to the guidelines and instructions set.
- (2) GeniUL does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by GeniUL; defects caused by misuse or use contrary to the Instructions supplied, or if the product is contaminated by improper handling or storage.
- (3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the manufacturing date. There are no other warranties that extend beyond those described in this document
- (4) GeniUL does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of GeniUL.
- (5) GeniUL does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labour, loss of time, inconvenience, and expenses for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death.
- (6) GeniUL reserves the right to replace or allow credit for any modules returned under this warranty.

5. OTHER INFORMATION

Agreement Terms

The purchase and use of this product signifies imply the conformity to the following terms:

(a) The use of this product may require a license, which is not provided by the purchase of this product. Users should obtain the license if required. The purchase and use of this package signifies the agreement of any purchaser or user of the enclosed components to the following terms:

1. The enclosed components may be used solely in accordance with the enclosed product information, where not include commercial use, testing, analysis or screening services for any third party in return for compensation. There is no license granted to use or incorporate the enclosed components for commercial use in a method under the respective intellectual property.
2. No warranty is made that the enclosed components and/or their use(s) do not infringe the rights of third parties.
3. The enclosed components are only for non-commercial use and may not be reused, refurbished, or resold.
4. Any other licenses, expressed or implied, are specifically disclaimed.
5. The purchaser and user of the enclosed components agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. The prohibitions of this Agreement

Terms may be enforced in any Court, and all the investigative and Court costs shall be recovered, including attorney fees, in any action to enforce this Agreement.

The patent rights and know-how related to the "Use of Phenanthridium Derivatives for Distinguishing Between Intact and Membrane-Compromised Cells Using Molecular Nucleic Acid-Based Techniques", including but not limited to United States Patent Application Serial No.: 12/280,946, filed August 8, 2007; European Patent Convention Application Serial No.: 07751663.1, filed February 28, 2007; and Japanese Patent Application Serial No.: 2008-557324, filed August 27, 2008 under a license agreement dated March 5, 2007. Further information on purchasing licenses under the above intellectual property rights may be obtained from QIAGEN GmbH, Business Development, bd@qiagen.com.

(b) GeniUL makes no warranty that this product and their use(s) do not infringe the rights of third-parties.

(c) GeniUL specifically disclaims any licenses, expressed or implied other than those expressly stated.

Other applications, in which propidium monoazide can be used, may be covered by patents issued and applicable in the United States, Japan and certain other countries. Because purchase of this product does not include a license to perform any patented application, users of these products may be required to obtain a patent license depending upon the particular application in which the product is used. The purchase of this product not contains any external license or right for their commercial use.

(d) The purchaser and user of the product agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. GeniUL may enforce the prohibitions of this Agreement Terms in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce theses Agreement Terms or any of its intellectual property rights relating to the Kit and/or its components.

This product is sold for research purposes;

It is not intended for food, drug, household, agricultural or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. Users should make independent decisions regarding completeness of the information based on all sources available. GeniUL shall not be held liable for any damage resulting from handling or contact with the above product.

6. CONTACT AND SUPPORT

If you have questions or experience problems with this or any other product of GeniUL, please contact our technical support staff (see details on www.geniul.com). Our scientists are committed to provide assistance quickly and effectively. We also would like to you contact us if you have suggestions to improve our product performance or the use of our products in new forms or applications.

GeniUL
Edifici GAIA
Rambla de Sant Nebridi, 22
08222 - Terrassa (Barcelona)
Spain
Tel: +34 936190312/ Fax: +34 932740232
geniul@geniul.com