

ExoSAP-IT

Product Number 78200	100 reactions
78201	500 reactions
78202	2000 reactions

STORAGE

Store at -20°C.

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.



GE imagination at work



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PRODUCT DESCRIPTION

ExoSAP-IT

Exonuclease I and Shrimp Alkaline Phosphatase in buffer.

STORAGE

Store at -20°C and keep on ice while pipeting. The enzymes are heat labile and will slowly lose activity at room temperature. Do not store in a frost-free freezer (the temperature rises above 0°C daily).

QUALITY CONTROL

All lots are functionally tested by USB® using radiolabeled-dATP and a control M13 clone single-stranded DNA template as described in the Sequenase™ PCR* Product Sequencing Kit (PN70170) protocol. Release specifications are based on sequence length, band intensity and sequence quality. The entire PCR product sequence must be readable on a standardized gel with less than 24 hours exposure. The sequence must also be free of background bands strong enough to interfere with sequence interpretation.

SAFETY WARNINGS AND PRECAUTIONS

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coats, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

See MSDS (Material Safety Data Sheet) on p. 8.

OVERVIEW

ExoSAP-IT™[†] prepares PCR products for numerous applications, such as sequencing by either radioactive or fluorescent detection methods. When PCR amplification is complete, any unconsumed dNTPs and primers remaining in the PCR product mixture will interfere with these methods. ExoSAP-IT utilizes two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase, to remove these unwanted dNTPs and primers. The Exonuclease I degrades residual single-stranded primers and any extraneous single-stranded DNA produced by the PCR. The Shrimp Alkaline Phosphatase hydrolyzes remaining dNTPs from the PCR mixture which would interfere with the sequencing reaction. ExoSAP-IT is added directly to the PCR product. The enzymes are active in the buffer used for PCR, hence no buffer exchange is required. After treatment, ExoSAP-IT is inactivated simply by heating to 80°C for 15 minutes.

ExoSAP-IT consists of one pipeting step and two incubations. Just add ExoSAP-IT to the PCR product and within 30 minutes be ready to sequence (Figure 1). All gel or column purifications, sedimentations, filtrations, beads and/or magnetic separations are eliminated. The method is designed to require a minimum of 'hands-on' time. Only simple pipette transfers are required, therefore many samples can be processed at once, either manually or with robotic devices.

After the clean-up procedure is complete the sequencing protocol of choice should be followed as described in the specific sequencing kit protocol booklet. When using automated sequencing reagents, it has been found that PCR product clean-up is not always necessary with dye-primer fluorescent sequencing methods, however, in many cases it is beneficial. When sequencing with fluorescent dye-terminators, it is essential that the PCR product be free of excess primers and dNTPs.

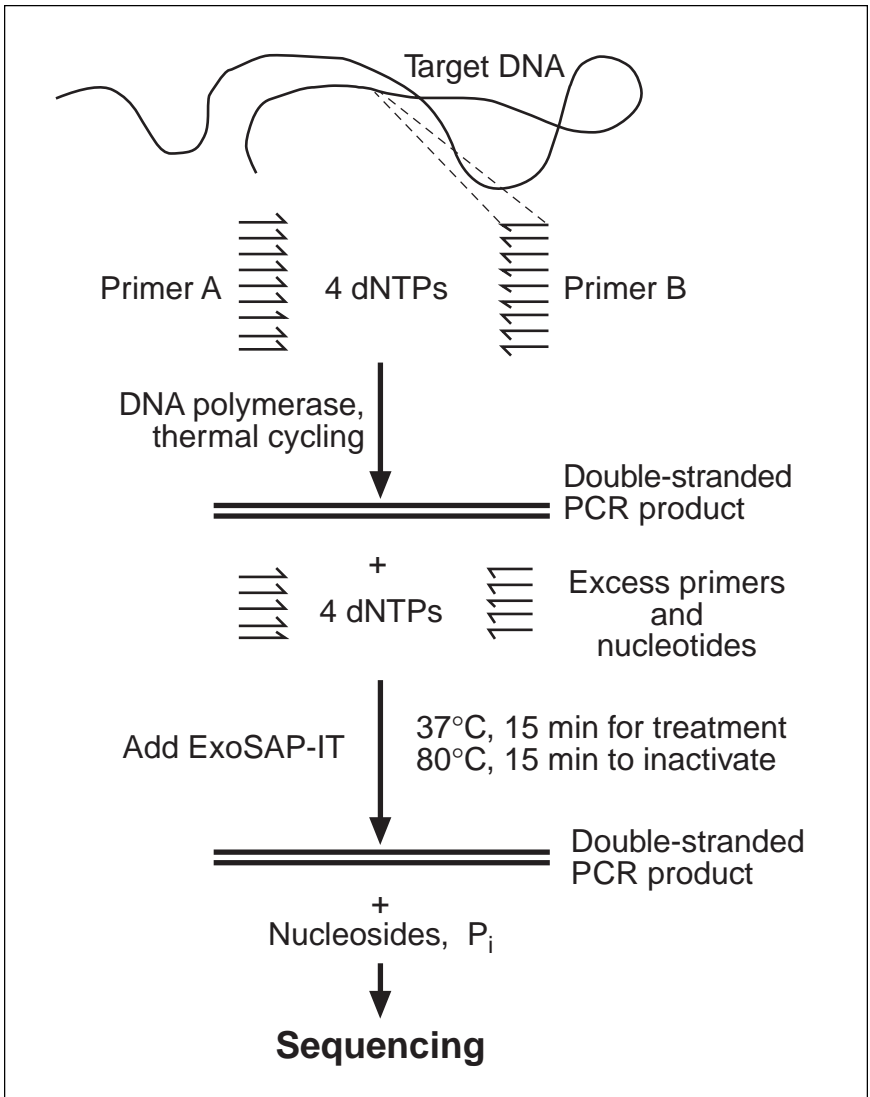


Figure 1: Schematic of the ExoSAP-IT method.

EXOSAP-IT PCR CLEAN-UP PROTOCOL

ExoSAP-IT may be used to clean-up PCR products ranging in size from less than 100bp up to over 20kb. No PCR product will be lost when using the ExoSAP-IT clean-up method. This is because the product is not subject to a purification method which may lead to sample loss, such as methods involving precipitations or columns.

1. PCR Product*:	5 μ l
ExoSAP-IT:	<u>2μl</u>
Total:	7 μ l

*Refers to the mixture after PCR amplification is complete.

Note: When treating 10 μ l or more of PCR product, increase the amount of ExoSAP-IT proportionally.

2. Mix and incubate at 37°C for 15 minutes. (It is convenient to do this step in a thermal cycler.)
3. Inactivate ExoSAP-IT by heating to 80°C for 15 minutes. (It is also convenient to do this in a thermal cycler.)
4. The DNA is now ready for direct sequencing using manual or automated methods.

RELATED PRODUCTS

Product	Application	Pack size	Product number
PCR Nucleotide Mixes	Functionally tested in long PCR	10mM, 500µl 25mM, 500µl	77212 77119
Sequenase Version 2.0 DNA Sequencing Kit	For non-cycle radioactive sequencing	100 templates	70770
Thermo Sequenase™ Cycle Sequencing Kit	For cycle radioactive sequencing	100 templates	78500
Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit	For cycle radioactive sequencing	50 templates	188403 With ³³ PddNTPs 79750 Without ³³ PddNTPs

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Sweden

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USA

Tel: 1 800 526 3593

Material Safety Data Sheet

Revision: 07/06/2000

Hazard information is provided for compliance with both the UK Chemicals (Hazard Information and Packaging) (CHIP) Regulations and the US Hazard Communication Standard (HCS)



**IDENTIFICATION OF THE
SUBSTANCE/PREPARATION
AND COMPANY**

PRODUCT NAME
ExoSAP-IT

PRODUCT CODE
78200 / 78201 / 78202

EEC NUMBER
None

SUPPLIER:

USB Corporation, 26111 Miles Road, Cleveland, OH 44128 216-765-5000

EMERGENCY CONTACT:

**Chemtrec 800-424-9300
Outside USA & Canada 703-527-3887**

**COMPOSITION/
HAZARDOUS
COMPONENTS**

<u>HAZARD</u>	<u>CAS NO.</u>	<u>%WT</u>	<u>TLV</u>	<u>CHIP R & S PHRASES</u>
Glycerol	56-81-5	~50.0%	ACGIH TLV - TWA: 10mg/m3 Total particulate OSHA TWA: 15mg/m3 (total dust)	R: 36/37/38 Irritating to eyes, respiratory system and skin S: 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
				S: 36/37 Wear suitable protective clothing and gloves.

HAZARDS IDENTIFICATION

CHIP Irritant
HCS Irritant

FIRST-AID MEASURES

EYES: Flush with water for 15 minutes. Seek medical advice if irritation persists.
SKIN: Flush with water, then wash thoroughly with soap and water. Remove contaminated clothing and wash before reuse. Seek medical advice if irritation persists. **INHALATION:** Move to fresh air. If breathing is difficult, give artificial respiration. Keep victim quiet and warm. Seek medical attention immediately.
INGESTION: Drink water and seek immediate medical advice. Avoid alcoholic beverages. Never give anything by mouth to an unconscious person.

FIRE-FIGHTING INFORMATION

Flash point = 193°C (379.4°F) Autoignition temperature = 400°C (752°F) Explosion Limits = Lower - 1.1 Upper - Not available. Extinguishing media - Media suitable to extinguish the supporting or surrounding fire. For small fires only: use carbon dioxide, dry powder or foam. For large fires: Evacuate the area and notify the Fire Department immediately. Special Fire Fighting Procedures - Wear self-contained breathing apparatus and full protective gear. Use water spray to cool any fire-exposed containers. Unusual fire or explosion hazards - Contact with strong oxidizing agents may produce an explosion.

ACCIDENTAL RELEASE MEASURES

Wear suitable protective clothing including lab coat, safety glasses and gloves to clean small releases. Ventilate the area and stop the leak if it can be done without risk, dilute with water before mopping or take up with sand, earth, or other absorbing material. Place material in a suitable dry, leak-proof waste container. Avoid contact of material with skin or eyes. Use adequate ventilation.

HANDLING AND STORAGE

Wash thoroughly after handling. Use with adequate ventilation. Wash clothing before reuse. Store material in a cool, dry place. Containers (even empty) may retain product vapors and residue. Store away from ignition sources and excess heat. Store away from incompatible materials including strong oxidizers, mixtures with hydrogen peroxide, potassium permanganate, calcium hypochlorite, nitric acid, sulfuric acid, perchloric acid and lead oxide.

PERSONAL PROTECTION

RESPIRATORY PROTECTION: A qualified industrial hygienist should evaluate the need for respiratory protection. Use respiratory protection approved by NIOSH and appropriate to the hazard. **VENTILATION:** Mechanical ventilation or local exhaust as needed to control exposure to dust, vapors or mists. **PROTECTIVE GLOVES:** Chemical-resistant gloves. **EYE PROTECTION:** Chemical-resistant goggles.
OTHER: Lab coat or other long-sleeved garment to limit skin exposure. Access to a safety shower and eye wash.

Continued on next page.

PHYSICAL AND CHEMICAL PROPERTIES

Boiling Point = 288°C Solubility = Miscible in water Melting Point = 20°F
 Decomposition Temp. = 290°C Vapor Pressure = .0025 mm Hg@ 5 Specific Gravity = 1.26 Vapor Density = 3.17 (H₂O = 1) Percent Volatile = No data available
 Evaporation Rate = No data available Formula = C₃ H₈ O₃ Appearance = Clear, viscous liquid; odorless to bland odor.

STABILITY AND REACTIVITY

Glycerol is stable under normal conditions. Avoid strong oxidizing agents including mixtures with hydrogen peroxide, potassium permanganate, calcium hypochlorite, nitric acid, sulfuric acid, perchloric acid and lead oxide. Contact with Sodium Hypochlorite and Hypochlorous acid may cause an explosion.

TOXICOLOGICAL INFORMATION

Effects of overexposure to Glycerol: EYES: Contact may cause irritation and slight corneal injury. SKIN: Prolonged contact may cause irritation and/or allergic reaction. INHALATION: No known toxicity, but excessive fumes may cause irritation if inhaled. INGESTION: May cause irritation of gastrointestinal tract and diarrhea. Additional Information: May cause slight or transient irritation to eyes and skin. Has caused moderate irritation in dermal (rabbit) studies. Low single and repeated dose toxicity. Ingesting large quantities may cause nausea and vomiting. Irritation, mutation, reproductive effects and toxicity data for Glycerol is listed in RTECS under MA805000. See RTECS for complete information. Toxicity data (Glycerol): Oral Mouse LD₅₀ = 4090 mg/kg Oral rat LD₅₀ = 12600 mg/kg. Emergency and First Aid Information: See First Aid Measures above.

ECOLOGICAL INFORMATION

No information available

DISPOSAL CONSIDERATIONS

Dispose of material in accordance with applicable local, state, and federal regulations.

TRANSPORTATION INFORMATION

US DOT / IATA - No information available.

REGULATORY INFORMATION

TSCA - CAS # 56-81-5 is listed on the TSCA inventory This material is not listed under TSCA Section 12b SARA 302 - This material does not have an RQ or TPQ. SARA 313 - This material is not reportable under 313. EXPOSURE LIMITS - ACGIH TLV TWA: 10mg/m³ Total particulate OSHA PEL TWA: 15mg/m³ (total dust)

This data sheet is based upon information believed to be reliable. The company makes no statement or warranty as to the accuracy or completeness of the information contained herein which is offered for your consideration, investigation and verification. Any use of the information contained in this data sheet must be determined by the user to be in accordance with appropriate applicable regulations.

*Exonuclease I/Shrimp Alkaline Phosphatase method of use covered by one or more of the following US patents: 5,756,285 and 5,741,676. ExoSAP-IT patent pending.

Sequenase is a trademark of GE Healthcare Bio-Sciences Ltd or its subsidiaries.

*The Polymerase Chain Reaction (PCR) is covered by patents owned by Roche Molecular Systems and F. Hoffmann-La Roche Ltd.

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