EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX PCR / DNA Clean-Up Purification Kit

Kit for purification of PCR products / DNA after enzymatic reactions

Cat. no. E3520

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Table of Contents

Content	50 preps E3520-01	150 preps E3520-02	Storage/Stability
Buffer DX	1.8 ml	5.4 ml	15-25°C
Orange DX	24 ml	72 ml	15-25°C
Wash DX1	30 ml	90 ml	15-25°C
Wash DX2	36 ml	108 ml	15-25°C
Elution	9 ml	27 ml	15-25°C
DNA Binding Columns	50	3 x 50	15-25°C
Protocol	1	1	

Introductory Notes

NOTE 1 · Kit Specification. The kit is suitable for fast cleanup of up to 25 µg of DNA fragments from PCR and other enzymatic reactions (sizes from approximately 100 bp to over 15 kb). This kit selectively removes primers below 40 nt and double-stranded DNA below 20 bp. However, common short by-products of not optimal or problematic PCR, known as primer-dimers, also consist of double-stranded DNA. They are produced from self-annealed and extended primers and co-migrate on a gel along with unincorporated single-stranded DNA primers. These double-stranded DNA artefacts co-purify with an expected PCR product, if their length exceeds 20 bp. If the removal of primer-dimers is necessary, we recommend PCR reaction optimization and/or agarose gel electrophoresis followed by isolation of PCR product using our GeneMATRIX Agarose-Out Purification Kit.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg. The maximum volume of the column reservoir is 650 µl.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature. In case of occasional buffer ingredients precipitation, simply warm up in 37°C water bath, until clarified.

NOTE 4 · Maintaining Good Working Practice. All solutions should be kept tightly closed to avoid evaporation and resulting concentration changes of buffer components. To obtain high quality DNA, stick carefully to the protocol provided below.

The kit provides spin columns and buffers for silica-membrane-based purification of DNA fragments from PCR and other enzymatic reactions. Purified DNA can be used in routine molecular biology applications such as PCR, sequencing and cloning. Protocol offers a simple bind-wash-elute procedure. Procedure removes primers, nucleotides, enzymes, mineral oil, salts, and other impurities from DNA samples. Binding buffer is added directly to the PCR sample or other enzymatic reaction, and the mixture is then applied to the minicolumn where nucleic acids adsorbs to the silica membrane in the high-salt conditions provided by the buffer. Impurities are washed away and pure DNA is eluted in a small volume of elution buffer.

Equipment and reagents to be supplied by the experimenter.

1. Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes.

Protocol

1. Apply 30 µl of activation **Buffer DX** onto the spin-column (do not spin) and keep it at room temperature till transfering mixture (point 3) to the spin-column.

   - *Addition of Buffer DX onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*
   - *The membrane activation should be done before starting isolation procedure. The minimum activation time is 5-15 min.*

2. Add 2 volumes of orange-coloured **Orange DX** buffer to 1 volume of the DNA sample and mix.

   - *For example, add 200 µl of Orange DX buffer to 100 µl DNA sample.*
   - *Maximum volume of a DNA sample can not exceed 200 µl. The minimum volume of DNA sample is 40 µl. If the sample volume is less than 40 µl, bring to a volume of 40 µl with sterile distilled water.*

3. Apply the mixture to the **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

4. Add 500 µl of **Wash DX1** buffer and spin down at 11 000 x g for 1 min.

5. Remove spin-column, pour off supernatant, replace back spin-column.

6. Add 600 µl of **Wash DX2** buffer and spin down at 11 000 x g for 1 min.

7. Remove spin-column, pour off supernatant, replace spin-column.

8. Spin down at 11 000 x g for 1 min to remove traces of the **Wash DX2** buffer.

9. Place spin-column into new receiver tube (1.5–2 ml). Add 50–150 µl of **Elution** buffer to elute bound DNA.

   - *Addition of eluting buffer directly onto the center of the membrane improves DNA yield.*
   - *To improve recovery of larger DNA fragments (above 5 kb) it is recommended to elute with buffer heated to 80°C.*
   - *For elution of DNA the Elution buffer is highly recommended. The buffer is prepared using ultrapure water with trace addition of buffering compounds. The Elution buffer will not interfere with subsequent DNA manipulations, such as DNA sequencing, ligation or restriction digestion, among others.*
   - *It is possible to reduce the volume of eluting buffer below 50 µl (no less than 20 µl). However, recovery of DNA will gradually decrease.*

10. Incubate spin-column/receiver tube assembly for 1 min at room temperature.

11. Spin down at 11 000 x g for 1 min.

12. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

Safety Information

Buffer DX

Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331 If swallowed: Rinse mouth. Do not induce vomiting.
P303+P361+P353 If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P405 Store locked up.

Orange DX

Danger

H302+H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H317 May cause an allergic skin reaction.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P284 [In case of inadequate ventilation] wear respiratory protection.
P301+P312 If swallowed: call a poison center/ doctor if you feel unwell.
P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+P313 If skin irritation or rash occurs: get medical advice/attention.

Wash DX1

Warning

H226 Flammable liquid and vapour.
H302+H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312 If swallowed: call a poison center/ doctor if you feel unwell.
P302+P352 If on skin: wash with plenty of water.
P304+P340 If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Wash DX2

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+P235 Store in a well-ventilated place. Keep cool.
P337+P313 If eye irritation persists: get medical advice/ attention.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

			ISOLATION OF DNA																			
			E3600	E3585	E3540	E3580	E3510	E3545	E3560	E3555	E3525	E3520	E3595	E3535	E3500	E3565	E3515	E3570	E3575	E3530	E3550	E3551
			MICELLULA DNA[2]	GRAM PLUS & YEAST GENOMIC DNA	AGAROSE – OUT DNA	BACTERIAL & YEAST GENOMIC DNA	BIO – TRACE DNA	BASIC DNA	BONE DNA	CELL CULTURE DNA	FOOD EXTRACT DNA	PCR / DNA CLEAN-UP	PLANT & FUNGI DNA	AGROBACTERIUM PLASMID DNA	PLASMID MINIPREP DNA	QUICK BLOOD DNA	SHORT DNA CLEAN-UP	SOIL DNA	STOOL DNA	SWAB-EXTRACT DNA	TISSUE DNA	TISSUE & BACTERIAL DNA
			AVAILABLE NUMBER OF ISOLATION (PREPS)																			
			50 150	25 100	50 150	50 150	25 100	50 150	25 50	50 150	25 100	50 150	50 150	50 150	50 150	50 150	25 100	50 100	50 100	25 100	50 150	50 150
DNA	GENOMIC	BACTERIA		●		●																●
		YEAST		●		●																
		CELL CULTURE								●											●	●
		PLANT											●									
		FUNGI											●									
		PLANT RICH IN POLYSACCHARIDES[1]											●									
		BLOOD														●						
		SOIL																●				
		STOOL																	●			
		SWAB																		●		
		ANIMAL TISSUES																			●	●
		FFPE TISSUE SECTIONS																			●	●
		RODENT TAILS																			●	●
		HAIR																			●	●
		INSECTS																			●	●
		URINE																			●	●
		BONE							●													
		BIOLOGICAL TRACES					●															
		FOOD									●											
	PLASMID	BACTERIA						●						●	●							
		YEAST				●																
	ISOLATION FROM AGAROSE GELS				●			●														
	PURIFICATION OF PCR PRODUCTS / DNA AFTER ENZYMATIC REACTIONS		●					●				●					●					

All kits contain buffers WASH in ready to use form

1. Additionally required lyse CT buffer (E0324)
2. Kit for creation of emulsions and subsequent DNA purification.

GeneMATRIX is synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures.

Novel binding and washing buffers are developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids. Matrix is conveniently pre-packed in ready-to-use spin-format. Unique chemical composition of the matrixes along with optimized construction of spin-columns improve the quality of final DNA or RNA preparation. To speed up and simplify isolation procedure, the key buffers are colour coded, which allows monitoring of complete solution mixing and makes purification procedure more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Such DNA or RNA can be directly used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybrydization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX PCR / DNA Clean-Up Purification Kit is designed to isolate DNA fragments, which were subjected to or obtained as a result of various modifications and reactions: PCR products, restriction digests, after kinasing, dephosphorylation, end-trimming/repair, ligation, enzymatic or chemical modification, among others.

Fragment of sizes from approximately 100 bp to over 15 kb can be obtained in ultrapure form. Effectively removed are contaminants such as: ethidum bromide, primers (below 40 nt), short double-stranded DNA (below 20 bp), RNA, Taq DNA Polymerase, Pfu DNA Polymerase, endo- and exonucleases, DNA-binding and modifying proteins, BSA and other enzymes/proteins, lipids, endotoxins, dyes, detergents, nucleotides, radio- and chemical labels, EDTA, problematic restriction and ligation inhibitors, buffers and salts. GeneMatrix is especially optimized toward binding DNA molecules over the very wide range of sizes: from 100 bp to over 15 kb as well as toward removal of problematic inhibitors of restriction and ligation of DNA. Coloured binding buffer is very helpful in simultaneous processing of multiple samples. DNA is then eluted in low salt buffer, e.g.: Tris-HCl, TE or water. Isolated DNA is ready for downstream applications without the need for ethanol precipitation.

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