Micropure®-EZ Enzyme Removers

■ Remove restriction and other enzymes from DNA in 30 seconds

Micropure-EZ provides an easy, rapid means of removing restriction and other enzymes from double-stranded (ds) DNA. The membrane in the device has a high affinity for protein but not for dsDNA. In a single 30-second spin, enzymes from the reaction mix are selectively adsorbed and DNA is recovered in a vial — undiluted and enzyme-free. If concentration or desalting of the sample is required, Microcon® centrifugal filter devices may be substituted for the filtrate vial, providing for DNA purification, concentration and desalting in a single unit.

Unlike precipitation or "bind and elute" purification, Micropure-EZ needs little hands-on time. This is especially useful when processing multiple samples. Also, DNA recovery is not affected by the binding capacity of an adsorption matrix. Micropure-EZ will deliver minimum 85% recovery of dsDNA, higher with a quick 5-10 µL rinse.

Features

Fast
- 30-second enzyme removal at 14,000 x g

Easy to use
- 1. Load; 2. Spin; 3. Done.
- Negligible hands-on time
- One-step DNA purification
- Concentration or desalting with addition of Microcon

Safe
- Avoid phenol

High DNA recovery
- Over 85%

Convenient
- Ideal for multiple sample processing
- Microcentrifuge-compatible
- 14,000 x g rated

Compare with Alternatives

Although enzyme removal by heat inactivation after a reaction is simple and inexpensive, many enzymes cannot be completely inactivated by this method. Also, heat degradation of DNA may occur. Precipitation techniques and "bind and elute" protocols, requiring a series of steps, are labor-intensive. Extraction followed by precipitation techniques requires the use of organic solvents, which, if carried over, can inhibit subsequent enzyme reactions. By comparison, Micropure-EZ is simple, fast and assures high recovery. Just load the enzymatic reaction directly into Micropure-EZ and spin for 30 seconds. dsDNA, free of enzymes, is efficiently recovered in the filtrate (see Figure 1).

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Concentrating and Desalting

When DNA concentration or buffer exchange are desired in addition to purification, all three steps can be performed in a single device by adding a Microcon centrifugal filter devices (see Figure 2). The combination of Micropure-EZ and Microcon allows DNA to be simultaneously separated from enzymes and concentrated. By adding buffer to the concentrated DNA, a second "diafiltration" spin can desalt the sample or exchange buffer. Result: DNA recoveries are superior to traditional techniques. No organic solvents or lengthy protocols are needed.

Specifications

Maximum initial sample volume
250 µL

Holdup volume, membrane, support
< 5 µL

Maximum relative centrifugal force
14,000 x g
(500 x g for Microcon YM-100)

Dimensions

Diameter
10.7 mm

Length

Micropure-EZ plus vial	40.9 mm
with Microcon	49.2 mm

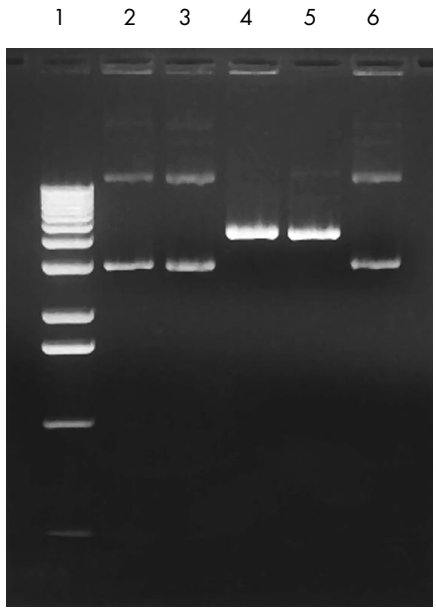

Figure 3. Micropure-EZ removes enzyme activity without binding DNA or affecting subsequent DNA manipulation.

Lane 1: 1 kb DNA ladder

Lane 2: Uncut, untreated pBR322

Lane 3: pBR322 spun through Micropure-EZ (No detectable changes in DNA: No nick, cuts, etc.)

Lane 4: pBR322 incubated with 10 units of Pst I for 1 hr at 37 °C

Lane 5: pBR322 in the restriction buffer spun through Micropure-EZ, then digested with 20 units of Pst I for 1 hr at 37 °C (No enzyme inhibitors leaching from the membrane)

Lane 6: Reaction mixture containing 100 units of Pst I passed through Micropure-EZ and incubated with pBR322 for 18 hrs at 37 °C (No detectable enzyme activity).

Selection Guide

Intended use	Recommended device
Enzyme removal only	Micropure-EZ
Enzyme removal and DNA concentration/desalting	Micropure-EZ filter unit only and Microcon YM-30 or YM-50

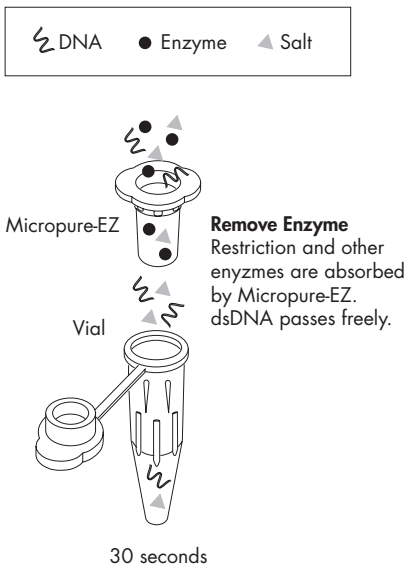

Figure 1. Enzyme removal with Micropure-EZ

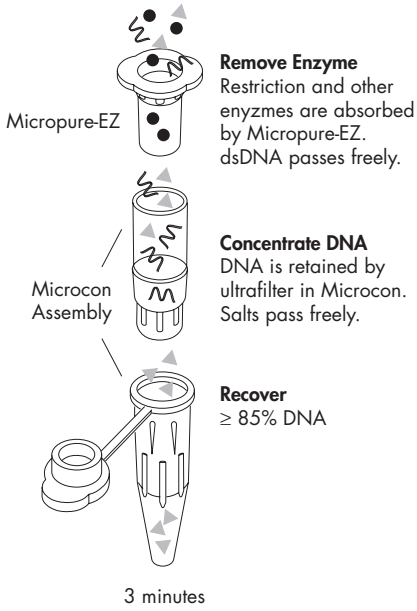

Figure 2. Simultaneous enzyme removal and concentration, buffer exchange or desalting, with Micropure-EZ and Microcon

Enzymes Removed by Micropure-EZ

In tests, all enzymes were removed from solutions containing DNA or RNA (in the case of RNase A). Five µg of bovine serum albumin were also present during removal of restriction enzymes. Removal was indicated by undetectable or inconsequential enzyme activity in filtrate (< 0.08% residual in the case of T4 polynucleotide kinase).

Enzyme	Challenge
AMV reverse transcriptase	50 U
Calf intestinal alkaline phosphatase	10 U
DNase I (bovine pancreas)	10 U
Exonuclease III (*E. coli*)	100 U
MMLV reverse transcriptase	600 U
Mung bean nuclease	50 U
Proteinase K (Amresco)	5 µg
T4 DNA ligase	2,000 U*
T4 DNA polymerase	15 U
T4 polynucleotide kinase**	50 U
Taq DNA polymerase	5 U
Terminal deoxynucleotidyl transferase	45 U
Acc I	50 U
Apa I	100 U
*Bam*H I	100 U
Bcl I	50 U
Bgl I	50 U
*Bsi*W I	70 U
*Bss*H II	20 U
*Bst*N I	50 U
Dpn I	100 U

Enzyme	Challenge
*Eco*R I	100 U
Hae III	100 U
Hinc II	50 U
Hind III	100 U
Hpa I	25 U
Kpn I	50 U
Mbo I	25 U
Mlu I	50 U
Nco I	50 U
Nde I	100 U
*Ngo*M I	50 U
Nhe I	25 U
Not I	50 U
Nru I	50 U
Pst I	100 U
Sac I	100 U
Sac II	100 U
Sal I	100 U
Sca I	50 U
Sph I	25 U
Sst I	50 U
Xho I	100 U

* New England Biolabs unit definition

** T4 polynucelotide kinase is not recommended with oligonucleotides (dsDNA only). The results suggest that this kinase mediates the binding of oligos to the membrane in Micropure-EZ device, causing oligo loss.

Enzymes Not Removed by Micropure-EZ

In tests, Micropure-EZ did not remove the indicated number of units of the listed enzymes. It may be effective in removing a lower number of units.

Enzyme	Challenge
Bacterial alkaline phosphatase	0.6 U
DNA polymerase I (Klenow)	20 U
Exonuclease I	50 U
Pfu DNA polymerase	2.5 U
Vent™ DNA polymerase	4 U
Shrimp alkaline phosphatase	1 U
RNase A (bovine pancreas)	1 µg
*Apa*L I	10 U
Bgl II	50 U
*Bso*B I	50 U
Cla I	25 U

Enzyme	Challenge
Eae I	15 U
*Eco*R V	50 U
Hinf I	50 U
Msp I	100 U
Pvu I	25 U
Pvu II	50 U
*Sau*3A I	20 U
Sfi I	50 U
Sma I	25 U
Xba I	50 U

T4 RNA ligase is not recommended. Results suggest this ligase mediates binding of nucleic acids to the membrane in Micropure-EZ causing sample loss.

Ordering Information

Description	Membrane MW Cut-Off	Nucleotide Cut-off Double-stranded	Qty/Pk	Catalogue No.
Micropure-EZ Enzyme Removers			8	42528
			24	42549
			100	42530
Micropure-EZ Enzyme Removers			8	42531
Filter units only (for use with Microcon)			24	42532
			100	42533
Microcon Centrifugal UF Filter Devices				
(includes 2 vials with each device)				
Microcon YM-3	3,000	10	8	42420
			24	42403
			100	42404
Microcon YM-10	10,000	20	8	42421
			24	42406
			100	42407
Microcon YM-30	30,000	50	8	42422
			24	42409
			100	42410
Microcon YM-50	50,000	100	8	42423
			24	42415
			100	42416
Microcon YM-100	100,000	125	8	42424
			24	42412
			100	42413

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