StrataClean Resin

INSTRUCTION MANUAL

Catalog #400714 and #400715
Revision B.0

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400714-12

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StrataClean Resin

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StrataClean Resin

MATERIALS PROVIDED

Material Provided	Amount	
	Catalog #400714	Catalog #400715
StrataClean resin	3 ml	9 ml

Revision B.0

INTRODUCTION

StrataClean™ resin is a phenol-free technique for DNA purification. The solid phase silica-based resin contains hydroxyl groups that react with proteins in much the same manner as the hydroxyl group of phenol. DNA can be rapidly and efficiently separated from all restriction enzymes and some modifying enzymes by treatment with the resin. DNA extracted with StrataClean resin may be used directly for sequencing or can be precipitated and used for manipulations such as ligations and restriction digests.

Note *Do not use this kit for RNA purification.*

PROTOCOL FOR EXTRACTION OF RESTRICTION AND MODIFYING ENZYMES

StrataClean™ resin is an excellent substitute for phenol for extracting all restriction enzymes as well as some modifying enzymes (see Table IV). StrataClean resin is guaranteed for use with only Stratagene enzymes. Refer to Table I for a list of enzymes that can be used with the resin. Best results are obtained if the sample has to the following specifications:

Maximum reaction volume*	250 µl
Maximum (final) enzyme concentration	10% (vol/vol)
Maximum salt concentration	150 mM

* For volumes exceeding 250 µl, use 10% v/v StrataClean resin.

Protocol

The volume of resin required for each extraction is approximately equal to the square root of the reaction volume. See Table II for the volume of resin required.

1. Vortex the slurry to completely resuspend the settled resin.

2. Using a sterile tip, pipette the required volume of slurry into the reaction mix. Vortex the mix for 15 seconds.

3. Incubate the mix at room temperature for one minute.

4. Spin the mix in a microfuge at 2000× *g* or more for one minute.

5. Using a pipette tip, carefully remove the supernatant containing the DNA to a fresh microfuge tube.

   Note *When removing the supernatant, remove a volume equal to the original reaction volume.* ***DO NOT attempt to remove any more supernatant, as this may result in carry-over of resin that could interfere with subsequent enzymatic reactions.***

6. Repeat steps 1-5. In a few cases, a third extraction is required (see Table III).

7. Precipitate the DNA by conventional techniques.

Double digests

If the enzymes do not use the same restriction buffer, digest first with one enzyme. Remove the enzyme with the resin using the protocol above. Increase reaction volume by 50% with H_2O, add new buffer and digest with the second enzyme.

If the enzymes use the same restriction buffer, digest with both enzymes at the same time. Be sure the final glycerol concentration does not exceed 5% (v/v). Remove the enzymes with the resin according to the protocol above.

PROTOCOL FOR THE PREPARATION OF MOUSE-TAIL DNA FOR SCREENING TRANSGENIC GENES USING STRATACLEAN RESIN

StrataClean resin can be used in place of phenol:chloroform to purify mouse genomic DNA from tail clippings. The resulting DNA is of a quality suitable for use in hybridizations.

Protocol

1. Digest mouse-tail clippings (each approximately 6 mm long) in 750 µl of 1X STE, 1% SDS and 25 µl of 10 mg/ml proteinase K for 3 hours at 55°C or alternatively at 37°C overnight.

2. After digestion, add 30 µl of StrataClean resin, invert several times to mix and spin for 10 minutes in a microfuge at 14,000 rpm.

3. Extract the supernatant and transfer to a new tube. Add 750 µl of ice-cold isopropanol and invert to mix. A DNA "hairball" should form.

4. Pellet the DNA for 4 minutes in a microfuge at 14,000 rpm. Remove the supernatant and allow the DNA to air dry.

5. Resuspend the DNA with 250 µl of TE buffer (10 mM Tris-HCl; 1 mM EDTA, pH 7.8).

Preparation of DNA for Probing

1. Add 10 µl of resuspended DNA to 100 µl of 0.35 M NaOH to denature the DNA.

2. Prepare the membranes for blotting. Immediately before blotting, neutralize the denatured DNA with 40 µl of 0.75 M ammonium acetate.

3. Blot 70 µl of DNA onto the membrane and apply 50 mm Hg vacuum to the blotter until the sample disappears from the wells. Wash the wells with 70 µl of 2 M ammonium acetate and apply 50 mm Hg of vacuum to the blotter until the wash disappears from the wells.

4. Allow the membrane to air dry, then UV crosslink at 900 µjoule in the Stratalinker UV crosslinker for approximately 1 minute, or alternatively oven bake for 1–2 hours.

APPENDIX: TABLES

Table I: Restriction Enzymes Requiring 2 Extractions

Aat II	Dpn I	Mlu I	Spe I
Acc II	Dra I	Mnl I	Sph I
Acc III	Dra II	Msp I	Ssp I
Afl II	Dra III	Mst II	Sty I
Aha II	Eae I	Nae I	Stu I
Alu I	Eag I	Nar I	Xba I
Apa I	EcoR I	Nci I	Xho I
ApaL I	EcoR II	Nco I	Xho II
Ava I	EcoR V	Nru I	Xma I
Avr II	Esp I	Nsi I	Xmn I
Bal I	Fok I	PpuM I	
BamH I	Fsp I	Pst I	
Ban I	Hae II	Pvu I	
Ban II	Hae III	Pvu II	
Bbv I	HglA I	Rsa I	
Bcd I	Hha I	Rsr II	
Bgl I	Hinc III	Sac I	
Bsm I	Hind III	Sac II	
Bsp106	Hinf I	Sal I	
Bsp128	Hpa I	Sau3A I	
BspM I	Hpa II	Sau96 I	
BssH II	Hph I	Scr FI	
BstX I	Kpn I	Sma I	
Dde I	Mbo I	SnaB I	

Table II: List of Slurry Volumes Required for Each Extraction

Reaction volume (µl)	Volume of slurry required for each extraction (µl)
10	3
20	5
50	7
100	10
150	12
200	14
250	16

Table II: List of Enzymes Requiring 3 Extractions

Acc I*	Ava II	Cla I	Fnu4H I	Sca I	Taq I*

* For complete extraction, final enzyme concentration in the reaction mix must be 8% (v/v) or less.

Table IV: Modifying Enzymes that can be Removed Using StrataClean Resin

Enzyme	Method for Using StrataClean Resin
CIAP	0.01 U to 1.0 U in reaction volumes up to 100 µl. Perform 3 extractions with 20 µl of StrataClean resin each time.
DNase I	0.4 U/µl final concentration in reaction volumes up to 250 µl. Perform 3 extractions.
Exo III	4 U/µl final concentration in reaction volumes up to 250 µl.
Taq DNA polymerase	≤5 U in up to 100 µl.

Table V: Enzyme Concentrations Tested with StrataClean Resin

Below is a list of enzymes tested with StrataClean resin. 86 of 90 can be quantitatively removed from 10–250 µl reactions with two resin extractions. The remaining six (bolded) require three extractions (see also footnote).

Enzyme	Final conc.	*Enzyme*	Final conc.	*Enzyme*	Final conc.
Aat II	0.2 U/µl	Dra III	0.45 U/µl	Nde I	1.2 U/µl
Acc I**	**0.4 U/µl**	Eae I	0.4 U/µl	Not I	0.7 U/µl
Acc II	0.3 U/µl	Eag I	0.1 U/µl	Nru I	0.5 U/µl
Acc III	0.3 U/µl	EcoR I	4 U/µl	Nsi I	0.6 U/µl
Afl II	0.6 U/µl	EcoR II	0.15 U/µl	PpuM I	0.15 U/µl
Aha II	0.8 U/µl	EcoR V	1.2 U/µl	Pst I	4 U/µl
Alu I	**0.3 U/µl**	**Esp I**	**0.25 U/µl**	**Pvu I**	**0.2 U/µl**
Apa I	1.2 U/µl	**Fnu4H I***	**0.2 U/µl**	Pvu II	1.25 U/µl
Apa LI	1 U/µl	Fok I	0.5 U/µl	Rsa I	2 U/µl
Ava I	1 U/µl	Fsp I	0.25 U/µl	Rsr II	0.1 U/µl
Ava II*	**0.8 U/µl**	Hae II	1 U/µl	Sac I	0.7 U/µl
Avr II	0.05 U/µl	Hae III	1 U/µl	Sac II	2 U/µl
Bal I	0.03 U/µl	HgiA I	0.5 U/µl	Sal I	1.5 U/µl
BamH I	4 U/µl	Hha I	5 U/µl	Sau3A I	1 U/µl
Ban I	1 U/µl	Hinc II	0.5 U/µl	Sau 96 I	1.5 U/µl
Ban II	0.7 U/µl	Hind III	4 U/µl	**Sca I***	**1 U/µl**
Bbv I	2 U/µl	Hinf I	1 U/µl	Scr FI	0.6 U/µl
Bcd I	0.8 U/µl	Hpa I	0.4 U/µl	Sma I	1.2 U/µl
Bgl I	0.8 U/µl	Hpa II	0.5 U/µl	SnaB I	0.4 U/µl
Bsm I	0.5 U/µl	Hph I	0.2 U/µl	Spe I	0.6 U/µl
Bsp 106	1 U/µl	Kpn I	1.6 U/µl	Sph I	0.35 U/µl
Bsp 128	0.2 U/µl	Mbo I	1.4 U/µl	Ssp I	1.2 U/µl
BspM I	0.15 U/µl	Mlu I	0.8 U/µl	Sty I	1 U/µl
BssH II	0.2 U/µl	Mnl I	0.1 U/µl	Stu I	1.2 U/µl
BstX I	0.5 U/µl	Msp I	5 U/µl	**Taq I****	**2 U/µl**
Cla I*	**0.8 U/µl**	Mst II	0.1 U/µl	Xba I	2.8 U/µl
Dde I	1.1 U/µl	Nae I	0.5 U/µl	Xho I	1.4 U/µl
Dpn I	0.5 U/µl	Nar I	0.9 U/µl	Xho II	0.5 U/µl
Dra I	1.6 U/µl	Nci I	0.8 U/µl	Xma I	2 U/µl
Dra II	0.8 U/µl	Nco I	0.7 U/µl	Xmn I	1 U/µl

* Three extractions

** Three extractions. For complete extraction, final enzyme concentration should be 8% (v/v) or less.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.