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18% SPRI reagent

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We use this protocol and it's working

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Abstract

This protocol explains how to prepare 18% SPRI beads that can be used instead of AMPure XP beads.
Note that as with any home-brew reagent, batches can show different behavior and should be tested (with a DNA ladder) prior to use.

Guidelines

This reagent, if stored in the refrigerator and covered from light, can last for up to 2 years (and potentially longer) in our experience.
Keep the stock at 4C in the dark and make smaller working aliquots after the beads are well suspended.

Materials

Magnetic racks for different tubes/plates:
- 50ml magnetic separation rack (e.g. NEB: S1507S or V&P Scientific: VP 772FB-2)
- 1.5/2.0ml magnetic separation rack (e.g. DynaMag-2 Magnet; ThermoFisher Scientific: 12321D)
- 96-well plate separation rack (ring magnet works well for automation, e.g. 96S Super Magne;, Alpaqua Engineering: A001322)
- 96-well plate separation rack (bar magnet or other magnets for 96 well plates work well for manual multichannel pipetting, e.g. Invitrogen: 12027 or Maxi Scientific: 4E-MRK100296)

1 Liter bottle, e.g. Nalgene Square PET Media Bottles with Closure, ThermoFisher Scientific: 342040-1000

	item	vendor	part number
	Sera-Mag Speedbead carboxylate -modified [E3] magnetic particles	Cytiva	65152105050250 (15ml)
	PEG-8000	Millipore-Sigma	89510
	5M NaCl	Millipore-Sigma	S5150
	1M Tris-HCl	Invitrogen	15568025
	0.5M EDTA	Millipore-Sigma	03690
	water	Millipore-Sigma	W4502
	optional: GeneRuler Ultra Low Range DNA ladder	Thermo Scientific	FERSM1211

Prepare TE for bead washing and resuspension

	reagent	volume	final concentration
	1M Tris-HCl	10	10 mM
	0.5M EDTA	2	1 mM
	water	988	
	TOTAL	1000	

Safety warnings

Mix Sera-Mag Speedbeads well before washing and SPRI reagent before using or aliquoting.

Before start

This protocol is for 750ml 18% SPRI reagent (using a single bottle of 15ml of the Sera-Mag Speedbeads).
If you make less, proportionally downscale.

Wash beads

1 For a total of 750ml 18% SPRI reagent, use the entire 15ml bottle of Sera-Mag Speed beads carboxylate-modified and vortex vigorously to remove thick aggregates of beads; transfer into a 50ml Falcon tube.

2 Add 20 ml TE to rinse the storage bead bottle, mix and transfer to the Falcon tube; mix well by vortexing, then briefly centrifuge tube to collect liquid from inside the lid (e.g. by swinging your arm or short low centrifugation)

3 Place tube in magnet to collect beads on tube wall, this takes about 10 minutes or until liquid is clear

4 Remove supernatant with serological pipet and add between 20 and 35 ml TE, mix well to wash beads more by vortexing.

5 Repeat steps 3 and 4 for a total of 2 washes, and resuspend washed beads in 15ml TE.

preparation of 18% SPRI reagent

6 Weigh out 135 g of PEG-8000 into a 1 Liter bottle.

7 To the PEG-8000 add below reagents

	reagent	volume in ml	final concentration
	5M NaCl	150	1 M
	1M Tris-HCl	7.5	10 mM
	0.5M EDTA	1.5	1
	washed beads from Step 5	15	0.1% solid particles

and fill up with water to 750 ml.
Mix well and let stand until all PEG particles are dissolved; intermittent shaking is helpful

8 Label with date and store in a dark box (or cover with aluminum foil) in the refrigerator.

9 This reagent is comparable to AmPure XP beads and can be used as recommended for the original product.
(The recommended volume is 1.8x for standard PCR cleanup to retain dsDNA > 100bp and clean away shorter primers and adapters etc)

Optional: preparing of 38% SPRI reagent

10 If a SPRI reagent is needed that allows to retain shorter fragments and to work in smaller volumes (e.g. to fit in a specific plate for automation), prepare a 38% SPRI reagent by following the above, but use 285 g of PEG-8000.
The 38% SPRI reagent can be used in equal volume to the cleanup PCR reactions from e.g. ancient DNA library preparations.

Recommended: testing of new reagent batch

11 Use 5 µl (2.5 µg total) of GeneRuler Ultra Low Range DNA ladder
Add 95 µl water
add 180 µl 18%SPRI (1.8x as recommended for AMPure XP)
(or 100 µl 38% SPRI)
mix by vortexing or pipetting
incubate 5 min at room temperature
spin and place on appropriate magnet for 4 minutes or until liquid is clear (it takes longer for the more viscous 38%SPRI)
wash beads while tubes remain on magnet with fresh 80% ethanol twice
spin, put back on magnet and remove remaining ethanol with pipet
air dry for 1min at room temperature
elute in 20 µl TE by resuspending the beads by pipetting
spin, place on magnet and let separate for 1min
collect elution into new tube

12 visualize on BioAnalyzer or TapeStation together with diluted original ladder of comparable starting amount
18% SPRI: the 100 bp band should be less strong and the shorter fragments should be gone
38% SPRI: the 50 bp should be as strong as before the cleanup and 35 bp should be more faint, 25 bp band should be gone.

Protocol references

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