Sage Hi-Bead™

HMW DNA Concentration Kit

PN# HBK-0012

Workflow Guide

Kit Contents

Binding Buffer	**4°C**	**1.7 ml**
Hi-Bead Suspension	**4°C**	**1.1 ml**
1X Te buffer (10mM Tris-HCl, pH 8, 0.1mM EDTA)	**room temp.**	**1 ml**

User-supplied Reagents and Equipment

Ethanol, 100%, molecular biology grade
Water, molecular biology grade
Eppendorf DNA LoBind tubes, 1.5 ml **(Eppendorf Cat. No. 022431021)**
Magnetic rack for microcentrifuge tubes **(Permagen Cat. No. MSR1224B, or equivalent)**
HulaMixer™ Sample Mixer **(Invitrogen, Cat #15920D)**
1000µl, 200µl, 20µl pipettes and tips.
Wide-bore 200-300µl pipette tips

Storage

Store Sage Hi-Bead Suspension and Binding Buffer reagents at 4°C for up to 3 months. Bring to room temperature before use.

Intended Use

Sage Hi-Beads are used to concentrate and purify high molecular weight DNA from aqueous buffer samples. Sage Hi-Beads are intended for research use only.

INTRODUCTION

The Sage Hi-Bead protocol described below is specifically designed for concentration of intact HMW DNA molecules from samples with concentrations **below 10 ng/µl.** Such low concentration samples are commonly encountered using the Sage HLS-CATCH targeted large fragment enrichment procedures (see https://sagescience.com/applications/target-capture/). At input DNA concentrations below 10ng/µl, recoveries of 80% can be achieved as shown in the Table below.

Table: *Replicate Hi-Bead cleanups were performed on unsheared phage T4 DNA samples (166kb linear DNA) at two different input concentrations (0.5 and 5 ng/µl). Recovery was measured by Qubit HS assay.*

[DNA input] (ng/µl), 70µl total	**0.5**					**5**				
Replicate #	1	2	3	4	5	1	2	3	4	5
[Eluted DNA], (ng/µl), 20µl total	**1.41**	**1.38**	**1.35**	**1.35**	**1.44**	**15.40**	**15.60**	**15.30**	**15.30**	**15.90**
Recovery(%)	81	79	77	77	82	88	89	87	87	91
Ave. Recovery(%)	**79**					**89**				

Note: For each replicate assay the volumes are: DNA input, 70 µl; Bead Binding buffer, 70 µl; Hi-Beads suspension, 42 µl; 80% ethanol wash, 200 µl (each wash); Te elution, 20 µl.

HMW DNA CONCENTRATION/PURIFICATION PROTOCOL

1. Bring the **Binding Buffer** and the **Hi-Bead Suspension** to room temperature.

   NOTE: If there is precipitate in the Binding Buffer, briefly heat to 37°C and gently mix. Equilibrate the Binding Buffer to room temp before proceeding.

2. Using wide bore pipette tips and extremely slow pipetting (in order to avoid DNA shearing), transfer the sample into an Eppendorf LoBind 1.5ml tube. While transferring, estimate the volume of the sample using the pipette (for Sage Science's HLS- CATCH method, the volume of an elution module is typically between 70-80µl). **Throughout this protocol, the original DNA sample volume is defined as "one volume".**

   TIP: If possible, use an electronic pipette in manual pipetting mode to transfer input DNA using the lowest motor speed available. We recommend the Integra Viaflow Voyager or Viaflow II 300µl pipettes with wide-bore tips, or similar products. Alternatively, if using standard manual pipettes, try to aspirate and dispense the DNA sample by turning the volume adjustment knob of the pipette, to avoid any sudden movement of the sample during pipetting.

3. Add **one volume** of **Binding Buffer** to the side of the tube just above the level of the liquid sample. Mix the Binding Buffer and the DNA sample by gently flicking the tip of the tube 5 or 6 times.

   IMPORTANT! Do **not** mix by pipetting the solution up and down -- the DNA may stick to un-treated plastic after mixing with the Binding Buffer.

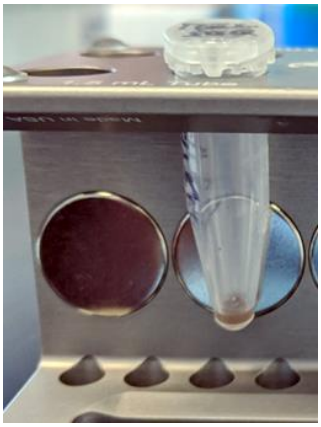
4. Place the tube in a HulaMixer and mix at room temperature (Reciprocal mode, 50°, 20 rpm) for 5 minutes.

5. Thoroughly resuspend the **Hi-Bead Suspension** by vortexing.

6. Add **0.6 volumes of Hi-Bead Suspension** to the side of the tube above the level of the sample. Mix by gently flicking the tip of the tube at least 5 times, so that the beads are evenly distributed throughout the sample mixture. Do not mix by pipetting.

7. Place the tube in a HulaMixer and mix at room temperature (Reciprocal mode, 50°, 20 rpm) for 10 to 30 minutes.

8. After mixing, do a brief spin (~1 second) on a minifuge to bring the bead mixture down to the bottom of the tube, and place it in the magnetic rack.



9. Wait until the sample clears -- this can take up to 5 minutes. Without removing the tube from the rack, use a pipette to remove and discard the supernatant, taking care not to disturb the pellet.

10. Without removing the tube from the rack, add **3 volumes** of freshly prepared 80% ethanol. Allow the ethanol wash to remain in contact with the bead pellet for at least 3 minutes. Use a pipette to remove and discard the supernatant, taking care not to disturb the pellet.

11. Repeat the 80% ethanol wash of step 10 with a second 3-volume aliquot of 80% ethanol. After pipetting off the bulk of the ethanol wash, remove the tube from the rack, spin briefly (~1 sec) in a minifuge, and place the tube back into the magnetic rack (with the bead pellet toward the magnet). Use a 20µl pipette to remove any remaining ethanol wash solution.

12. Add 1X Te buffer to the bead pellet to elute the concentrated DNA. Resuspend the bead pellet by gently flicking the tube.

    NOTE: The volume of Te to be used for elution will depend on the size of the input sample. For HLS-CATCH samples with 5-50 ng of total input DNA in a volume of 60-80µl, elution volumes of 10-40µl Te are recommended. Recovery efficiency is usually higher with larger elution volumes.

13. Incubate the resuspended Te+Hi-Bead mixture at 55°C for 10 minutes to elute the HMW DNA from the beads.

    NOTE: To reduce evaporative loss, the resuspended Te+Hi-Bead mixture can be transferred to a PCR tube and incubated in a thermocycler with a heated lid (lid at 65°C). Be sure to use wide-bore tips and slow pipetting for the transfer to avoid DNA shearing.

14. Remove the bead mixture from heat. Centrifuge briefly (~1 sec.) to collect all liquid to the bottom of the tube. Place tubes into the magnetic rack for 3-5 minutes, or until the solution above the bead pellet clears.

15. Without removing the tube from the magnetic rack, transfer the clear DNA solution from the tube to an Eppendorf LoBind tube for storage at 4°C.

    NOTE: We are currently unaware of manufacturers of wide-bore 20µl pipette tips, so when eluting with volumes ≤20µl, we suggest using a 20µl pipette tip that has been clipped with a clean scalpel blade to generate a wide-bore opening . For elution volumes >20µl, ThermoScientific™ ART™ 200XLG wide-bore 200µl tips (Fisher Scientific Cat# 21-402-159) are useful.