SOLiD® EZ Bead™ Enricher

Pub. Part no. 4443497 Rev. E **Rev. Date** October 2011

Note: For safety guidelines, refer to the "Safety" section in the *Applied Biosystems SOLiD® EZ Bead™ Enricher Getting Started Guide* (Part no. 4443496). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

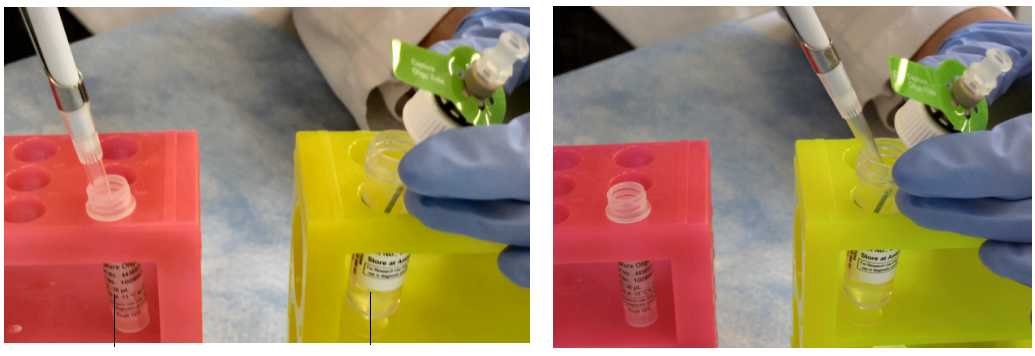
Prepare and install the reagents

1. Clean or prime the *SOLiD® EZ Bead™ Enricher* according to current instrument conditions (see "Clean the Enricher" on page 8).
2. Empty the waste container.
3. Install the *SOLiD® EZ Bead™ Enricher* Buffer Kit – Butanol. Ensure that the cap seals the bottle.

IMPORTANT! Confirm that the fan in the Enricher is on and circulating air.

4. Install Reagent Rack A.
5. Remove all used plastic consumables, if necessary.
6. Prepare the capture oligo:
   a. Transfer 1 mL of Capture Buffer (in *SOLiD® EZ Bead™ Enricher* Buffer Kit) from bottle B1 (pink label) to the *SOLiD® EZ Bead™ Enricher* Capture Oligo Tube (F Lime-green tube).

   b. Add 28 µL of Capture Oligo to the Capture Buffer in the Capture Oligo Tube (F Lime-green tube), then mix the solution.



   c. Install the tube and connect the tubing.

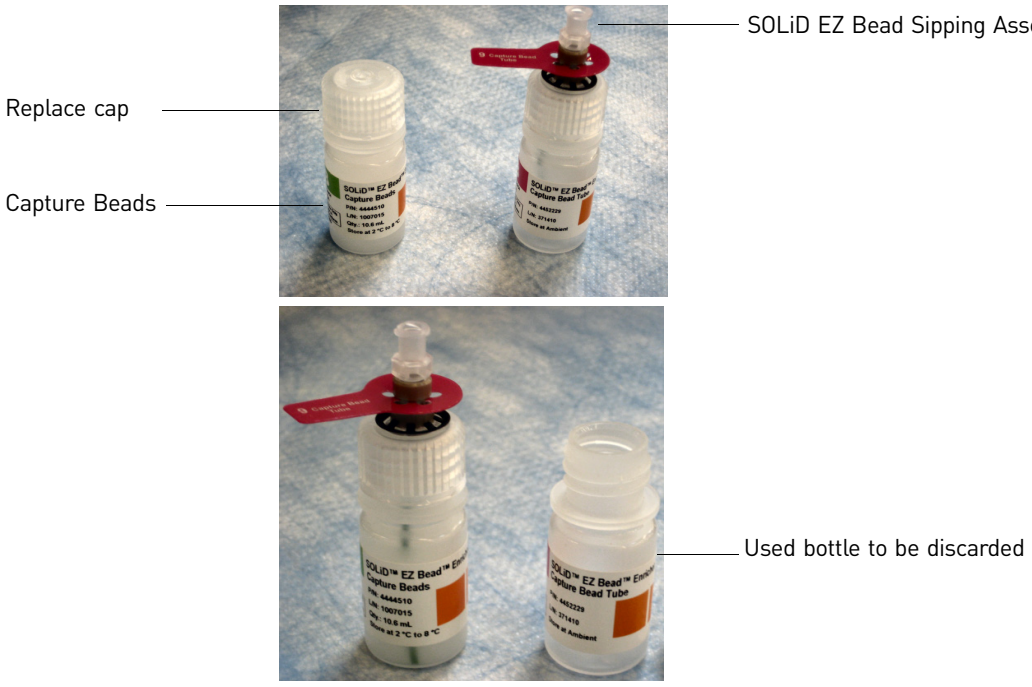
7. Pour the Buffer Kit-CS Solution 1 *Additive* into bottle B4 (yellow label) containing CS Solution 1 (in *SOLiD® EZ Bead™ Enricher* Buffer Kit).

8. Cap the bottles. Gently swirl reagent rack B for 10 seconds. Uncap the bottles, then install reagent rack B.

9. Install the following tubes:

Tubes to install	Matching numbers and colors on the tubing and tubes
__ *SOLiD® EZ Bead™ Enricher* QC Output Tube	5 Yellow
__ SOLiD EZ Bead Output Tube	3 Red
__ *SOLiD® EZ Bead™ Enricher* Unenriched Bead Bottle	4 Black
__ *SOLiD® EZ Bead™ Enricher* Release Tube	11 Purple
__ *SOLiD® EZ Bead™ Enricher* Hyb Tube	10 Tan
__ Connector on Cleaning Manifold	Connect to any other connector on the Cleaning Manifold

10. Replace the supplied cap on SOLiD EZ Bead Capture Bead Tube with a new SOLiD EZ Bead Sipping Assembly and install Capture Bead Tube on the instrument.



11. Install the *SOLiD® EZ Bead™ Enricher* Input Tube:

    a. Pour the emulsion into a tared Input Tube, then push the remaining emulsion in the SOLiD EZ Bead Amplifier pouch with a MicroAmp® Adhesive Film Applicator for greatest recovery of emulsion.

    b. Ensure that the weight of the emulsion is within range for the scale of emulsion: E10: 13 g-18 g, E20: 22 g-27 g, or E80/E120: 100 g-118 g. If the weight of the emulsion is not within range, then do *not* enrich the emulsion. Contact your Applied Biosystems Field Applications Specialist.

    c. Record the weight of the emulsion for later use: _____g.

    d. Install the Enricher Input Tube and connect the tubing.

12. Install the *SOLiD® EZ Bead™ Enricher* Column and connect the tubing.

13. Install the *SOLiD® EZ Bead™ Enricher* Filter and connect the tubing.

Run the Enricher

1. Power on the instrument.
2. Open the SOLiD EZ Bead software by double-clicking the icon 

. The Enricher control window displays.
3. Click the scale-selection button to select the scale in the control window (**E10**, **E20**, **E80**, or **E120**).
4. Enter the emulsion weight in grams (g). If the weight of the emulsion is not within the specified range shown on the control window, then do not start the run. Contact your Applied Biosystems Field Applications Specialist.
5. Click the start-run button . A pre-run checklist displays.
6. Complete and check off each item in the checklist. You cannot proceed with the run until all items are checked off. You can exit the checklist by clicking the stop button . After you complete the checklist, ensure that the Enricher doors are closed. Click the check button at the bottom of the checklist to begin the run. After the run starts, the yellow "IN USE" on the front status panel is lit. The run time is approximately 3 to 4 hours, depending on the scale chosen. After the run, the Enricher stops automatically.

IMPORTANT!

- Do *not* close the laptop or put the laptop into hibernation mode. Closing the laptop or putting the laptop into hibernation mode can affect communication between the software and the instrument during a run.
- Only stop the run if the Enricher malfunctions. If the run is stopped, the sample cannot be recovered. To stop the run, press the stop button. If necessary, troubleshoot. Follow the procedure for cleaning the Enricher in the *Applied Biosystems SOLiD® EZ Bead™ Enricher Getting Started Guide* (Part no. 4443496). Then follow the EZ Bead emulsion process described in the *Applied Biosystems SOLiD EZ Bead Emulsifier Getting Started Guide* (Part no. 4441486).
- Do not open either door of the Enricher during a run. If a door error occurs, the sample cannot be recovered. If the sample is lost because of a door error or other error, follow the procedure for cleaning the Enricher in the *Applied Biosystems SOLiD® EZ Bead™ Enricher Getting Started Guide* (Part no. 4443496). Then follow the EZ Bead emulsion process described in the *Applied Biosystems SOLiD® EZ Bead™ Emulsifier Getting Started Guide* (Part no. 4441486).

Concentrate and store the samples

IMPORTANT! Do *not* store the samples in the *SOLiD® EZ Bead™ Enricher* Bead Output Tube, the *SOLiD® EZ Bead™ Enricher* Unenriched Bead Bottle, or in the *SOLiD® EZ Bead™ Enricher* QC Output Tube.These tubes are not air-tight, so the samples can leak or evaporate.

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1. Remove the Output Tube, the QC Output Tube, and the Unenriched Bead Bottle from the Enricher. The Unenriched Bead Bottle contains a large volume of beads, which is normal.
2. Concentrate and store the *bead output*:
   a. Resuspend the beads, then transfer 2 mL of the bead output solution to a 2-mL LoBind Tube in a magnetic rack.
   b. After the solution clears, remove and discard the supernatant.
   c. Repeat step a. Do *not* remove the supernatant.
   d. Vortex and pulse-spin the 2-mL LoBind Tube.
   e. (Optional) Check the quality of the output beads using a flow cytometer and the SOLiD Bead QC Kit (Part no. 4456038). Refer to "Supplementary Procedures" in the *SOLiD® EZ Bead™ Enricher Getting Started Guide*.

IMPORTANT! Perform FACS QC before 3'-end modification. Modification of the 3' ends negatively affects FACS analysis.

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f. Store the samples at 4 °C until use, or proceed to "Modify the 3′ ends (E10)" on page 4, "Modify the 3′ ends (E20)" on page 5; "Modify the 3′ ends (E80)" on page 6; or "Modify the 3′ ends (E120)" on page 7.

3. Concentrate and store the *QC output*:

   a. Resuspend the beads, then pipet all of the bead output solution (1 mL) into a 1.5-mL LoBind Tube.

   b. (Optional) Check the quality of the QC output beads using a flow cytometer and the SOLiD Bead QC Kit (Part no. 4456038). Refer to "Supplementary Procedures" in the *SOLiD® EZ Bead™ Enricher Getting Started Guide*.

   IMPORTANT! Perform FACS QC before 3′-end modification. Modification of the 3′ ends negatively affects FACS analysis.

   c. Store the samples at 4 °C until use.

4. Concentrate and store the *unenriched beads*:

   a. Swirl the Unenriched Bead bottle to resuspend the beads.

   b. Pour the unenriched beads into a 50-mL conical tube.

   c. Centrifuge the 50-mL tube for 5 minutes at 1462 × g.

   d. Discard all but 2 mL of the supernatant.

   e. Vortex to resuspend the pellet, then transfer the bead suspension to a 2-mL LoBind Tube.

   f. (Optional) Check the quality of the unenriched beads using a flow cytometer and the SOLiD Bead QC Kit (Part no. 4456038). Refer to "Supplementary Procedures" in the *SOLiD® EZ Bead™ Enricher Getting Started Guide*.

   IMPORTANT! Perform FACS QC before 3′-end modification. Modification of the 3′ ends negatively affects FACS analysis.

   g. Store the samples at 4 °C until use.

Modify the 3′ ends (E10)

1. If the P2-enriched beads have been stored overnight or longer, sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System.

2. Prepare the 1X Terminal Transferase Reaction Buffer (500 µL buffer per sample):

Component	Volume per reaction (µL)
10X Terminal Transferase Buffer	55
10X Cobalt Chloride	55
Nuclease-free Water	390
Total	500

3. Add 1 µL of 50 mM Bead Linker to 49 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.

4. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

5. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer and transfer to a new 1.5-mL LoBind Tube. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

6. Resuspend the beads in 100 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.

7. Resuspend the beads in 178 µL of 1X Terminal Transferase Reaction Buffer.

8. Add 20 µL 1 mM Bead Linker solution.

9. Sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System, then pulse-spin.

10. Add 2 µL Terminal Transferase (20 U/µL), then vortex the solution. Pulse-spin.
11. Rotate the tube at 37 °C for 2 hours.
12. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
13. Resuspend the beads in 400 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
14. Resuspend the beads in 200 µL of 1X TEX Buffer.
15. Store the templated beads at 4°C in 1X TEX Buffer, or proceed to "Prepare and install slides and reagents" in the *Applied Biosystems SOLiD™ 4 System Instrument Operation Guide* (Part no. 4448379).

Modify the 3′ ends (E20)

1. If the P2-enriched beads have been stored overnight or longer, sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System.
2. Prepare the 1X Terminal Transferase Reaction Buffer (800 µL buffer per sample):

Component	Volume per reaction (µL)
10X Terminal Transferase Buffer	88
10X Cobalt Chloride	88
Nuclease-free Water	624
Total	800

3. Add 1 µL of 50 mM Bead Linker to 49 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.
4. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
5. Resuspend the beads in 200 µL of 1X Terminal Transferase Reaction Buffer and transfer to a new 1.5-mL LoBind Tube. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
6. Resuspend the beads in 200 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
7. Resuspend the beads in 356 µL of 1X Terminal Transferase Reaction Buffer.
8. Add 40 µL 1 mM Bead Linker solution.
9. Sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System, then pulse-spin.
10. Add 4 µL Terminal Transferase (20 U/µL), then vortex the solution. Pulse-spin.
11. Rotate the tube at 37 °C for 2 hours.
12. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
13. Resuspend the beads in 400 µL of 1X TEX Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
14. Resuspend the beads in 400 µL of 1X TEX Buffer.
15. Store the templated beads at 4°C in 1X TEX Buffer, or proceed to "Prepare and install slides and reagents" in the *Applied Biosystems SOLiD 4 System Instrument Operation Guide* (Part no. 4448379).

Modify the 3′ ends (E80)

1. If the P2-enriched beads have been stored overnight or longer, sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System.
2. Prepare the 1X Terminal Transferase Reaction Buffer (2.4 mL of buffer per sample):

Component	Volume per reaction (µL)
10X Terminal Transferase Buffer	264
10X Cobalt Chloride	264
Nuclease-free Water	1872
Total	2400

3. Add 4 µL of 50 mM Bead Linker to 196 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.
4. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
5. Resuspend the beads in 300 µL of 1X Terminal Transferase Reaction Buffer, then transfer the beads to a 2.0-mL LoBind Tube.
6. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
7. Resuspend the beads in 300 µL of 1X Terminal Transferase Reaction Buffer. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
8. Resuspend the beads in 1424 µL of 1X Terminal Transferase Reaction Buffer.
9. Add 160 µL of 1 mM Bead Linker solution.
10. Transfer 792 µL of bead solution to a new 2.0-mL LoBind Tube.
11. Sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System, then pulse-spin.
12. Add 8 µL of Terminal Transferase (20 U/µL) to each tube, then vortex the solution. Pulse-spin.
13. Seal each tube with Parafilm®, then rotate the tubes at 37 °C for 2 hours.
14. Pulse-spin the tubes, then pool the beads in one LoBind Tube.
15. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
16. Resuspend the beads in 400 µL of 1X TEX Buffer.
17. Place the tube in a magnetic rack for at least 1 minute, then discard the supernatant after the solution clears.
18. Resuspend the beads in 400 µL of 1X TEX Buffer.
19. Store the templated beads at 4°C in 1X TEX Buffer, or proceed to "Prepare and install slides and reagents" in the *Applied Biosystems SOLiD® 4 System Instrument Operation Guide* (Part no. 4448379).

Modify the 3′ ends (E120)

1. If the P2-enriched beads have been stored overnight or longer, sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System.
2. Prepare 5.0 mL of 1X Terminal Transferase Reaction Buffer (2.4 mL of buffer per sample):

Component	Volume per reaction (µL)
10X Terminal Transferase Buffer	550
10X Cobalt Chloride	550
Nuclease-free Water	3900
Total	5000

Note: The 1X Terminal Transferase Reaction Buffer should be clear. If the solution becomes colored, discard then prepare fresh buffer using a new lot of material.

3. Add 10 µL of 50 mM Bead Linker solution to 490 µL of 1X Low TE Buffer to prepare a 1 mM Bead Linker solution.
4. Place the tube of P2-enriched beads in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
5. Resuspend the beads in 600 µL of 1X Terminal Transferase Reaction Buffer, then transfer the beads to a 2.0-mL LoBind Tube.
6. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
7. Resuspend the beads in 600 µL of 1X Terminal Transferase Reaction Buffer.
8. Transfer 150 µL of beads to each of three 2.0-mL LoBind Tubes, for a total of four tubes.
9. Place the tube in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
10. Resuspend each tube of beads in 890 µL of 1X Terminal Transferase Reaction Buffer.
11. Add 100 µL of 1 mM Bead Linker solution to each tube and vortex to mix. Pulse-spin the beads.
12. Sonicate each tube of beads using the Covalent Declump 3 program on the Covaris® S2 System, then pulse-spin.
13. Add 10 µL of Terminal Transferase (20U/µL) to each tube, and vortex, then pulse-spin the beads.
14. Seal the tubes with Parafilm, then place the tubes on a rotator and rotate for 2 hours at 37°C.
15. Pulse-spin the tubes, then pool two tubes of beads into one DNA LoBind Tube. Pool the other two tubes of beads into one tube.
16. Place both tubes in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
17. Resuspend each tube of beads in 200 µL of 1X TEX Buffer. Pool the two tubes of beads into one tube.
18. Place both tubes in a magnetic rack for at least 1 minute. After the supernatant clears, remove and discard the supernatant.
19. Resuspend the beads in 400 µL of 1X TEX Buffer.

STOPPING POINT Store the templated beads at 4°C in 1X TEX Buffer, or proceed to "Prepare and install slides and reagents" in the *5500 Series SOLiD® Sequencers Operation Guide* (Part no. 4456991).

Clean the Enricher

IMPORTANT! Clean the Enricher after a run. Cleaning the Enricher after every run removes residual beads from the instrument. If a run was performed overnight or over the weekend, clean the Enricher as soon as possible within a 3-day period.

For detailed cleaning instructions, refer to the *Applied Biosystems SOLiD® EZ Bead™ Enricher Getting Started Guide* (Part no. 4443496).

Supplementary figures

Figure 1 Layout of *SOLiD® EZ Bead™ Enricher*: actual view

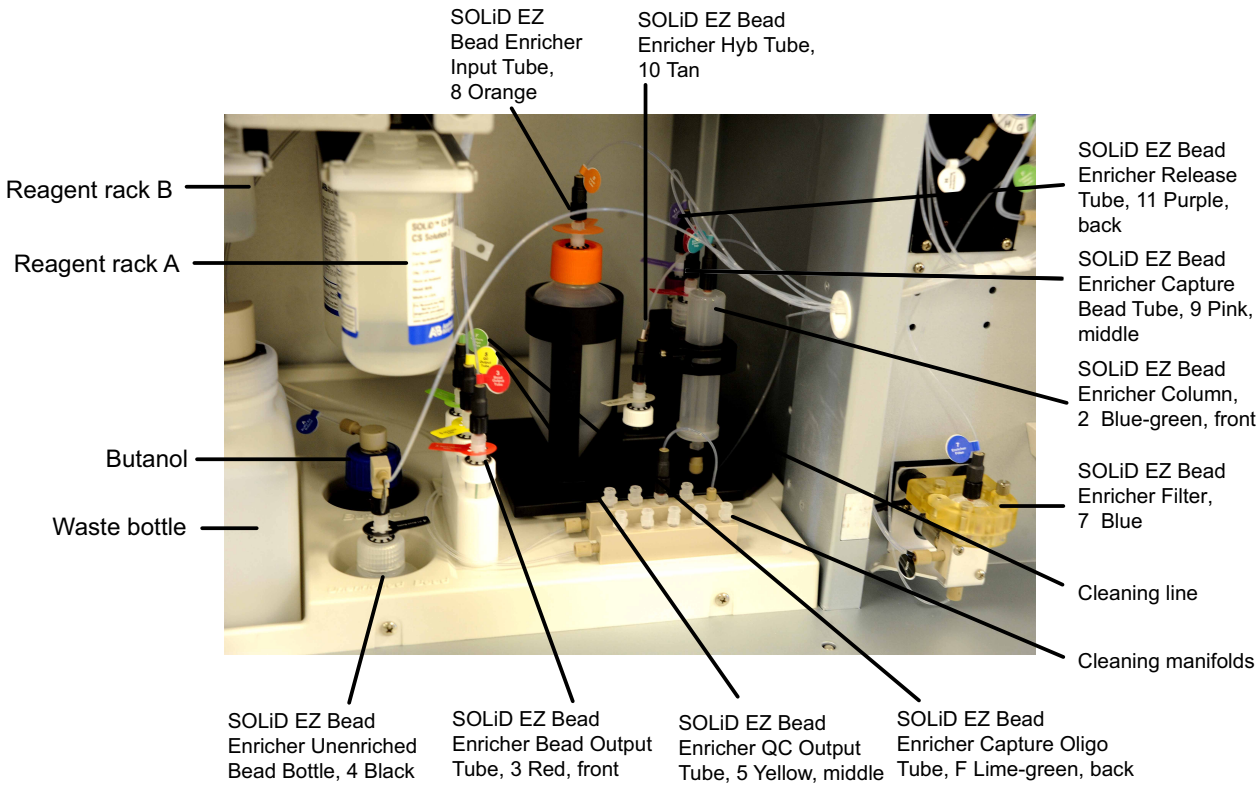
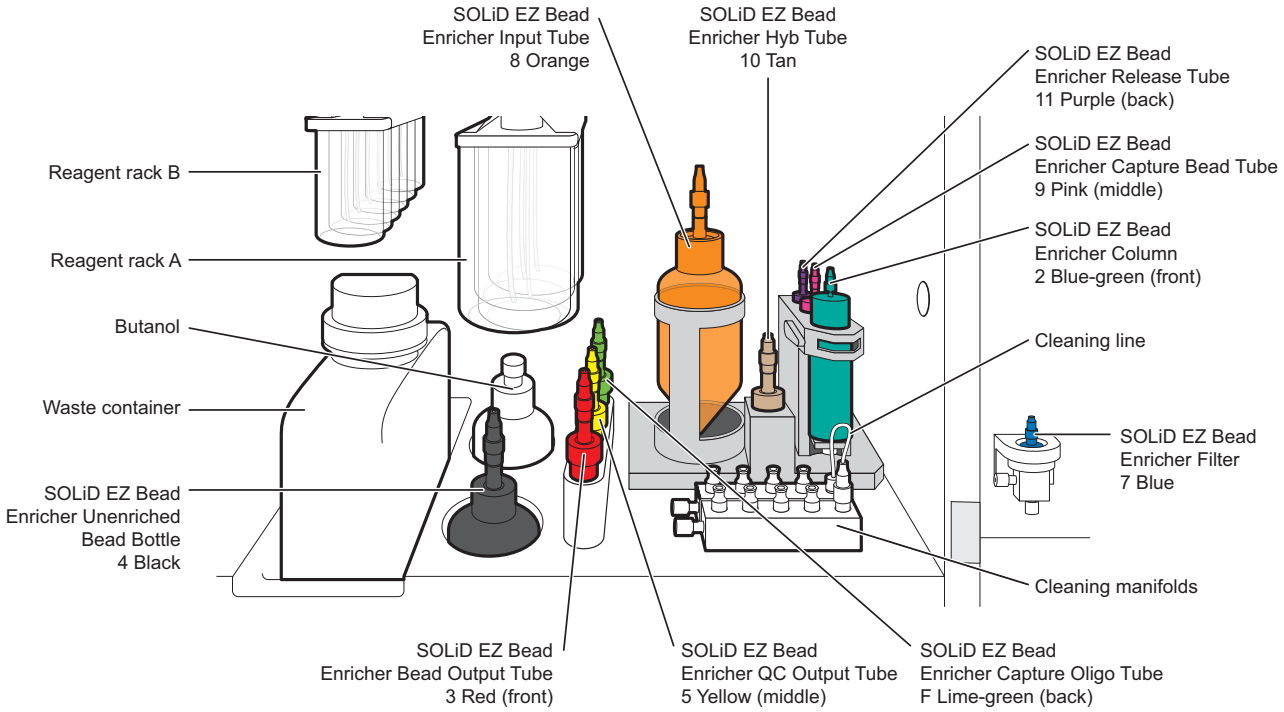


Figure 2 Layout of *SOLiD® EZ Bead™ Enricher*: schematic view.



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