

# SOLiD<sup>®</sup> EZ Bead<sup>™</sup> Amplifier

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**Note:** For safety guidelines, refer to the “Safety” section in the *Applied Biosystems SOLiD<sup>®</sup> EZ Bead<sup>™</sup> Amplifier Getting Started Guide* (PN 4443494). For every chemical, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Prepare the Amplifier Pouch

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**IMPORTANT!** Before using the stored Oil Master Mix, shake (by hand) the bottled mix for 30 seconds. Let the Oil Master Mix de-gas and reach room temperature for 1 hour in a dark location (such as in a cupboard or drawer).

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1. Prepare the Oil Master Mix (see [“Supplementary procedure: Prepare the Oil Master Mix”](#) on page 3.)
2. Pre-rinse the Amplifier E-Small (for E10, E20 scales) or E-Large (for E80, E120 scales) Pouch:
  - a. Unscrew the cap(s) of the nozzle(s), then add by serological pipette the appropriate volume of Oil Master Mix (E10, E20: 10 mL; E80, E120: 20 mL) to the Amplifier E-Small (E10, E20) or E-Large (E80, E120) Pouch.
  - b. Screw the cap(s) tightly on the pouch nozzle(s), then massage the pouch for 2–3 minutes to ensure that the Oil Master Mix coats the inner surface of the pouch.
  - c. Over a waste container, unscrew the cap(s), then drain the Oil Master Mix into the container. Gently squeeze the Amplifier Pouch to expel any remaining Oil Master Mix from the pouch.
  - d. Drain the Amplifier Pouch upside-down for at least 20 minutes on a layer of clean lint-free wipes (Kimwipes<sup>®</sup> Cleaning Wipes). After the pouch is drained, the pouch is ready for use.

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**IMPORTANT!** Do not re-use Oil Master Mix that was used to pre-rinse the Amplifier Pouches.

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- e. Screw the cap(s) tightly on the nozzle(s) until the Amplifier Pouches are ready for use. Use the pre-rinsed Amplifier Pouches the *same* day.
3. Insert an empty, *pre-rinsed* Amplifier E-Small Pouch (E10, E20) or an empty, *pre-rinsed* Amplifier E-Large Pouch (E80, E120) into the *outer* slots of the pouch filling station.
  4. Unscrew the cap(s) from the nozzle(s) of the pouch. Hold the funnel firmly in place in the nozzle.
  5. Slowly pour all of the emulsion into the pouch. Let the emulsion drip from the jar into the funnel for 30 seconds to recover as much of the emulsion as possible.
  6. Turn the crank of the filling station counter-clockwise until most air is expelled from the pouch.
  7. Remove the funnel. Screw the cap(s) tightly, then turn the crank clockwise to release the pouch.

## Set up and run the Amplifier

1. Pull the latch all the way up until it stops to open the door to the Amplifier.
2. Remove the pouch frame from the Amplifier, then place it on the bench top.

**Note:** For E10 and E20 scales only: Up to three pouches can be installed on a single amplifier.
3. Pull the foam away from the Amplifier pouch frame and install the pouch nozzle(s) in the holder.
4. Fit the Amplifier E-*Small* Pouch(es) into *any* of the slots or the Amplifier E-*Large* Pouch into the *outer* slots.
5. Release the foam so that it returns to its original position in the holder.

6. Place the pouch frame with installed pouch(es) back on to the Amplifier. Ensure that the pouch frame is properly seated on the Amplifier.
7. Close the door of the Amplifier, then push the lever *all the way down until it stops* to secure the door.
8. Open the **Amplifier** software.

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**IMPORTANT!** For libraries less than 200 bases pairs (bp) (including insert and adaptors), we recommend using the appropriate scale selection with the "sm" suffix [**E20sm, E80sm, E120sm**]. For the E10 scale, select the E10 scale option regardless of library size.

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9. On the control window, click the scale-selection button  to select the scale (**E10, E20, E20sm, E80, E80sm, E120, E120sm**), then click

the start button  to start the ePCR run.

After the ePCR is complete, the count-down timer displays "00:00:00" and "Execution completed".

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**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.
  - Do *not* open the door during the run. Opening the door compromises the sample because the sample is not properly amplified. If you open the door in error, stop the run, then prepare a new emulsion.
  - To stop the run, press the stop button . The left light in the control window is lit red, "Run aborted" is displayed in the control window; and the red "ERROR" on the control window is lit.
  - Restart the run on the Amplifier with a *new* emulsion.
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**WARNING! PHYSICAL INJURY HAZARD. Hot Surface.** Use care when working around this area to avoid being burned by hot components.

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10. After the run is complete, follow the appropriate step:
    - *Pouch is removed immediately after the run:* Remove the pouch from the Amplifier, then store the pouch at 4°C as soon as possible.
    - *Pouch is removed after an overnight run or the pouch is left unattended in the Amplifier:* The sample is stored automatically at 10°C. Remove the sample while the software is running and the instrument is at 10°C. For best results, do not store the post-PCR sample on the Amplifier for >36 hours.

**Note: For multiple E10 or E20 pouches, repeat step 9 for each pouch.**
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**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.

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11. After the pouch is removed from the Amplifier, click the scale select button  to return the Amplifier to ambient temperature.

## Supplementary procedure: Prepare the Oil Master Mix

**Note:** A single Amplifier Oil Pack Kit (PN 4457185) yields approximately 240 mL of Oil Master Mix, which is six 50-mL Falcon® tubes containing 40-mL of Oil Master Mix each. The 240-mL of Oil Master Mix is enough for:

- E10 scale: 8 runs (E10 emulsions + E10 bag rinse)  
– OR –
- E20 scale: 6 runs (E20 emulsions + E20 bag rinse)  
– OR –
- E80 scale: 2 runs (E80 emulsions + E80 bag rinse)  
– OR –
- E120 scale: 2 runs (E120 emulsions + E120 bag rinse)

1. Prepare the Oil Master Mix in a 50-mL Falcon® tube.

Item	Volume
Stabilizer 1	1.8 mL
Stabilizer 2	400 µL
Mineral oil	37.8 mL
<b>Total volume</b>	<b>40 mL</b>

- a. Use a 3-mL syringe to dispense 1.8 mL of Stabilizer 1 into the 50-mL Falcon® tube, as shown. To ensure no air bubbles are in the syringe, aspirate some reagent into the syringe and fully dispense it back into the reagent bottle. Then slowly aspirate the correct volume.
- b. Use a 1-mL syringe to dispense 400 µL of Stabilizer 2 into the 50-mL Falcon® tube, as shown. Make sure no air bubbles are in the syringe, aspirate some reagent into the syringe and fully dispense it back into the reagent bottle. Then slowly aspirate the correct volume.



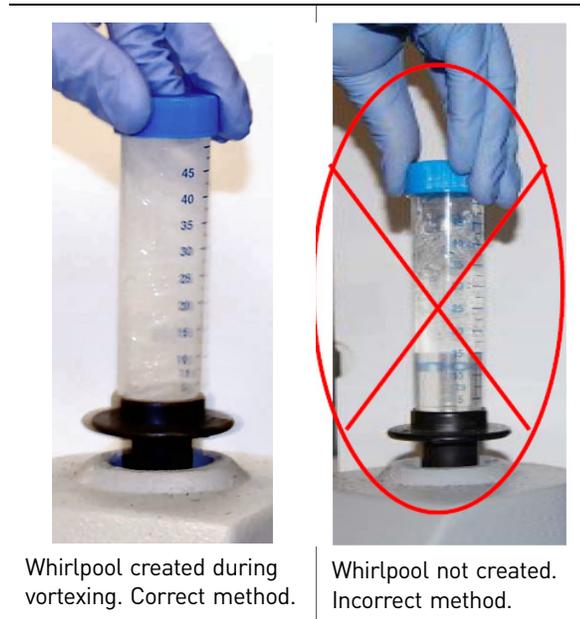
Stabilizer 1 (1.8 mL)



Stabilizer 2 (400 µL)

- c. Pour mineral oil (approximately 37.8 mL) into the tube that has Stabilizers 1 and 2 so that the final volume is 40 mL.

2. Cap the Falcon® tube. Use a vortexer to mix the solution so that a whirlpool is created that goes to the bottom of the tube. After the whirlpool has been created, continue to whirlpool-vortex the mixture for another 30 seconds.



3. Pour the mixture into the Oil Mix Bottle (in the Amplifier Oil Pack Kit).
4. Repeat steps 1 through 3 FIVE additional times, for a total of SIX times. Use a new 50 mL Falcon® tube each time. After performing steps 1 through 3 SIX times, you should have approximately 240 mL of Oil Master Mix in your Oil Mix Bottle.
5. Shake (by hand) the bottled mix for 30 seconds.
6. **If the Oil Master Mix will be used the same day:** De-gas at room temperature for at least 30 minutes in a dark location (such as in a cupboard or drawer).  
**If the Oil Master Mix will NOT be used the same day:** De-gas and store the Oil Master Mix at 4°C in a 4°C refrigerator.

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STOPPING POINT The Oil Master Mix can be stored at 4°C for up to two weeks.

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