

Hypothalamus Homogenization Using the Bullet Blender

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Materials

- [Bullet Blender](#)® for 5 mL tubes
- Homogenization Buffer
- [FoamBlocker](#) (Optional)
- [Lysis Kit](#) or [Lysis Beads](#)
 - PINK or RED Lysis Kit (from [PrecisionPak™](#) or purchased separately)
 - 2.0 mm Zirconium Oxide Beads in Eppendorf or GATOR tubes
- Sample — up to 1000 mg

Table 1. Proper sample, bead and buffer volume ratios for 5 mL tubes.

Lysis Kit and Bead Choices	Sample Volume	Bead Volume	Buffer Volume
PINK	Up to 300 mg	Pre-filled	0.5 - 1.2 mL
RED	300 - 1000 mg	Pre-filled	1.2 - 2.5 mL
2.0 mm Zirconium Oxide Beads	Up to 1000 mg	500 - 1000 µL	1.2 - 2.5 mL

Procedure

1. Use the pre-filled bead lysis kit tubes OR prepare a tube with the recommended volume of bead choices from the table above.
2. Add the appropriate volume of buffer according to the table above.
3. Prepare the sample by cutting it into small thin pieces and then transfer it into the buffer-filled tubes.
4. (Optional) To avoid excess foaming, add FoamBlocker up to 1-2% of the total volume of the homogenization buffer.
5. Close the tubes tightly and place into the Bullet Blender sample chamber. If using the Gold or Gold⁺ models, pre-cool the chamber before adding sample tubes.
6. Set the controls to speed 8, time 3 minutes then press Start.
Note: Using single-size beads instead of pre-filled lysis kits may require additional time.
7. After the run, remove the tubes from the instrument and visually inspect the samples. If homogenization is incomplete, homogenize for an additional 30 seconds, or repeat the homogenization step with a higher speed.
8. Using a pipette, transfer the homogenized samples into new tubes.
9. Proceed with downstream application.

Notes

This protocol does not specify a particular buffer – choose a buffer that is most appropriate for the downstream application or use the lysis buffer provided in a [PrecisionPak™](#), a simplified workflow solution which also includes a bead lysis kit, supplemental reagents for high quality nucleic acids isolations, and an optimized protocol for specific samples.

This protocol was developed using mouse tissue. Homogenization times, speeds, or beads may need to be optimized for other species, especially larger animals.