

QIAzol[®] Lysis Reagent

The QIAzol Lysis Reagent (cat. no. 79306) can be stored at room temperature (15–25°C) or at 2–8°C for at least 12 months.

For more information, please refer to the *QIAzol Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- Ensure that you are familiar with operating the TissueRuptor[®], TissueLyser LT, or TissueLyser II by referring to the respective user manual and handbook. For other disruption devices, refer to suppliers' guidelines.
 - Fresh, frozen, or RNA^{later}[®]/Allprotect[™] stabilized tissues can be used. Do not allow non-stabilized tissues to thaw during weighing or handling prior to disruption in QIAzol Lysis Reagent. Homogenized tissue lysates from step 2a or 2b can also be stored at –70°C for at least 1 month. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
1. Add QIAzol Lysis Reagent to an appropriate vessel for disruption and homogenization and subsequent centrifugation: 1 ml QIAzol Lysis Reagent per 100 mg tissue is required. The volume of tissue should not exceed 10% of the volume of QIAzol Lysis Reagent. If using the TissueLyser LT or TissueLyser II, add the stainless steel bead(s) of choice (1 x 5 mm or 2 x 7 mm) to the tube before the addition of QIAzol.
 2. Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue and place it into the QIAzol Lysis Reagent. Proceed immediately to step 2a (TissueRuptor) or 2b (TissueLyser).

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- 2a. Disruption using the TissueRuptor: Place the tip of the TissueRuptor disposable probe into the QIAzol Lysis Reagent, and operate the TissueRuptor at full speed until the tissue lysate is uniformly homogeneous (usually 20–40 s).
- 2b. Disruption with TissueLyser LT or TissueLyser II: Place tubes, or collection tube rack, in the respective TissueLyser adapter and operate the instrument according to instructions in the handbook.
3. Place the tube containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.
4. Add 0.2 ml chloroform per 1 ml QIAzol Lysis Reagent pipetted in step 1. Securely cap the tube containing the homogenate, and shake it vigorously for 15 s.
5. Place the tube containing the homogenate on the benchtop at room temperature for 2–3 min.
6. Centrifuge at 12,000 x g for 15 min at 4°C.
7. Transfer the upper, aqueous phase to a new tube. Be careful to avoid the interphase. Add 0.5 ml isopropanol per 1 ml QIAzol Lysis Reagent pipetted in step 1. Mix thoroughly by vortexing.
8. Place the tube on the benchtop at room temperature for 10 min.
9. Centrifuge at 12,000 x g for 10 min at 4°C.
10. Carefully aspirate and discard the supernatant.
11. Add at least 1 ml of 75% ethanol per 1 ml QIAzol Lysis Reagent pipetted in step 1. Centrifuge at 7500 x g for 5 min at 4°C.
12. Remove the supernatant completely, and briefly air-dry the RNA pellet.
13. Redissolve the RNA in an appropriate volume of RNase-free water. Clean up the RNA using the RNeasy® MinElute® Cleanup Kit or RNeasy Mini, Midi, or Maxi Kit.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

“RNAlater®” is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

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