User-Developed Protocol:

Preparation of RNA<sup>later™</sup> preserved tissues for histological studies

This procedure has been adapted by customers for use with RNA<sup>later™</sup> RNA Stabilization Reagent. It has not been thoroughly tested and optimized by QIAGEN.

The procedure can be used to prepare RNA<sup>later</sup> stabilized biopsies for histological and immunohistochemical studies.

Please be sure to read carefully the RNeasy<sup>®</sup> Mini Handbook (3<sup>rd</sup> Edition, June 2001, or later), the RNeasy Midi/Maxi Handbook (2<sup>nd</sup> Edition, June 2001, or later), or the RNeasy Protect and RNA<sup>later</sup> Handbook, including the detailed Protocol for RNA Stabilization in Tissues with RNA<sup>later</sup> RNA Stabilization Reagent, before beginning this procedure.

Procedure

1. **Remove stabilized tissue from the RNA<sup>later</sup> RNA Stabilization Reagent using forceps.**

2. **If only a portion of the stored tissue is to be used, place the tissue on a clean surface for cutting, and cut the tissue. Transfer the remaining tissue back into the RNA<sup>later</sup> RNA Stabilization Reagent for further storage, or store it at –80°C without the reagent.**

   RNA in the RNA<sup>later</sup> treated tissue is still protected while the tissue is processed at 18 to 25°C. This allows cutting and weighing of tissues at ambient temperatures. It is not necessary to cut the tissue on ice or dry ice or in a refrigerated room.

   The remaining tissue can be stored in RNA<sup>later</sup> RNA Stabilization Reagent for subsequent RNA isolation. The reagent preserves RNA for up to 1 day at 37°C, 7 days at 18 to 25°C, or 4 weeks at 2 to 8°C, allowing transportation, storage, and shipping of samples without ice or dry ice. Alternatively, the samples can be placed at –20°C or –80°C for archival storage.

3. **Wash the tissue twice for 15 min each time in PBS.**

   Once the RNA<sup>later</sup> RNA Stabilization Reagent is washed out, RNA in the tissue is no longer protected. However, this does not affect subsequent histological and immunohistochemical analyses.

4. **Remove the tissue from the PBS, and fix for 24 h in 10% buffered formalin.**
5. **Embed the tissue in paraffin or freeze in OCT medium** following standard procedures.
   
   Paraffin-embedded RNA<sub>later</sub> stabilized tissues can be used for immunohistochemical and routine hematoxylin and eosin staining.
   
   RNA<sub>later</sub> stabilized tissues frozen in OCT medium can be used for immunohistochemical and frozen-section hematoxylin and eosin staining.
   
   **Note:** After RNA<sub>later</sub> treatment, S-100 immunostain can appropriately stain nerve fibers but not melanocytes or Langerhan’s cells. In addition to S-100 antigen, other soluble antigens may not stain appropriately after treatment with RNA<sub>later</sub> RNA Stabilization Reagent.

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