Preparing Bone Samples for the RNAscope® Assay

Introduction

This Technical Note provides guidelines on how to decalcify bone samples for the RNAscope® Assay. Read the Safety Data Sheet (SDS) available on the website, and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves. For the latest services and support information, go to: **www.acdbio.com/support.**

Workflow

Part 1: Sample Preparation

Fix Bone Tissue

1. Immediately following dissection, fix bone tissue in 10% neutral buffered formalin (NBF) at **ROOM TEMPERATURE (RT)** for **16–32 HRS**.

NOTE: Fixation time may vary depending on bone tissue type and size.

2. Remove the fixative solution. Wash the sample with 1X PBS or TBS.

Decalcify Bone Tissue

1. Place the bone sample in 20 volumes of ACD Bone Decalcification Buffer relative to sample size (w/v).
2. Decalcify the sample at **4°C** for **SEVERAL HRS–2 WKS**, depending on the degree of mineralization and size of the specimen.
3. Remove the ACD Bone Decalcification Buffer, and replace with fresh Bone Decalcification Buffer every 2 DAYS.

NOTE: Samples are decalcified when the bone is soft and pliable, roughly two weeks for long bone. Preparing bone marrow requires optimization, and usually takes four hours.

3. Wash the decalcified bone tissue with 1X PBS for **5 MIN**. Repeat with fresh 1X PBS twice.
4. Store the sample in 70% alcohol in 1X PBS for **<24 HRS**, or continue with embedding the tissue.

Embed Bone Tissue

1. Use standard procedures to embed the sample in paraffin.

NOTE: You may store samples at **15–25°C** with desiccation. If you are storing samples for a long period of time (>1 year), we recommend storing at **2–8°C** with desiccation.

Part 2: Pretreatment

Apply RNAscope® Target Retrieval and Protease Plus

1. Follow the standard RNAscope® pretreatment protocol. Refer to the *FFPE Sample Preparation and Pretreatment User Manual* (Doc. No. 322452-USM).
2. (Optional) If partial tissue detachment occurs after the target retrieval step, replace RNAscope® Target Retrieval and Protease Plus with ACD Custom reagent (please contact Support team to get this reagent) at **40°C** for **30 MIN**.

NOTE: We do not recommend the alternative method for low expressing genes.

- Search through FAQs
- Submit a question directly to Technical Support

Obtaining Support

For the latest services and support information, go to:
https://acdbio.com/technical-support/support-overview.

At the website, you can:

- Access telephone and fax numbers to contact Technical Support and Sales

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7707 Gateway Blvd Suite 200, Newark, CA 94560 Phone 1-510-576-8800 Toll Free 1-877-576-3636
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