ACTIVE MOTIF®

Enabling Epigenetics Research

Tissue Prep for NGS Assays

Catalog No. 53185 (24 rxns)

(Version A1)

Contents

Overview

Product	Format	Catalog No.
Tissue Prep for NGS Assays	24 rxns	53185

Kit Components and Storage

The kit contains sufficient reagents for 24 reactions. The reagents in this kit have multiple storage temperatures. Please store components according to the storage conditions below. All reagents are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
Tissue Lysis Buffer - NGS Assays	24 mL	RT
40 µm Strainer	24 Strainers	RT
1X Wash Buffer	44 mL	4°C
Protease Inhibitor Cocktail	500 µL	-20°C

Additional Materials Required

- Dounce Homogenizer (1 mL, Active Motif Catalog Number 40401 or 15 mL 40415, or similar)
- Petri dish to prepare tissue sample in (30 mm to 60 mm diameter)
- Forceps to handle tissue
- Razor blade to prepare tissue
- Scale to weigh tissue sample
- Laboratory wipes such as Kimwipes
- Dry ice to keep tissue sample and tools chilled before processing
- Wet ice to keep tissue sample cool while preparing nuclei
- Method to count nuclei such as an automated cell counter or manual hemocytometer with Trypan Blue. We recommend automated cell counting methods such as the Countess from Thermo Fisher Scientific for this assay.
- 1.5 mL Low-bind Microcentrifuge tubes
- 2 mL Low-bind Microcentrifuge tubes
- CUT&Tag-IT® R-loop Assay Kit 53167 **OR** ChIC/CUT&RUN Assay Kit 53180

Tissue Prep for NGS Assays Protocol

This Kit is compatible with a range of tissue amounts, from 3 mg to 30 mg, and has been optimized for 10 mg tissue sample per reaction of heart, brain, liver, kidney or spleen tissue.

Tissue samples may be fresh or flash frozen on liquid nitrogen or dry ice. To flash freeze tissue samples for the assay at a later date, follow either the liquid nitrogen or dry ice steps below.

To Flash Freeze on Liquid Nitrogen

1. Excise the tissue from the animal and place in a microfuge tube.
2. Submerge in liquid nitrogen for 2 minutes.
3. Store at -80°C.

To Flash Freeze on Dry Ice

1. Excise the tissue from the animal and place in a microfuge tube.
2. Place tube on dry ice with ethanol for 15 minutes.
3. Store at -80°C.

Section A. Prepare Concanavalin A Beads (30 minutes)

Note: Concanavalin A Beads are included in CUT&Tag-IT® R-loop Assay Kit Cat No. 53167 and ChIC/CUT&RUN Assay Kit Cat No. 53180. Steps to prepare Concanavalin A Beads appear in the CUT&Tag-IT® R-loop Assay Kit and ChIC/CUT&RUN Assay Kit manuals.

Section B. Nuclei Extraction from Tissue Samples

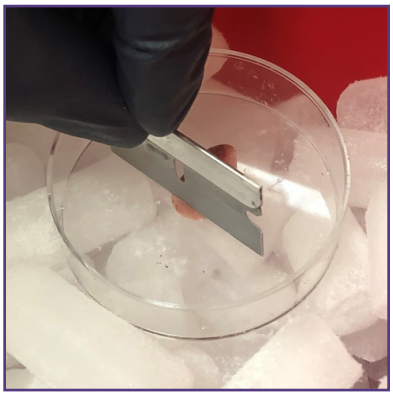
First: Prepare Tissue Lysis Buffer - NGS Assays with fresh Protease Inhibitor Cocktail. Per sample, add 10 µL Protease Inhibitor Cocktail to 990 µL Tissue Lysis Buffer - NGS Assays for 1 mL total volume. Keep the freshly prepared buffer on wet ice until it is used in the steps below.

Prepare 1 X Wash Buffer with fresh Protease Inhibitor Cocktail. Per sample, add 7 µL Protease Inhibitor Cocktail to 743 µL 1 X Wash Buffer for 750 µL total volume. Keep the freshly prepared buffer on wet ice until it is used in the steps below.

4. Place Dounce Homogenizer on wet ice to chill.
5. Place petri dish on scale and tare to be ready to weigh tissue sample.
6. Place the petri dish on dry ice to be ready for the tissue sample.
7. Ethanol spray the forceps and place on dry ice to chill.
8. Ethanol spray the razor blade and carefully wipe dry with clean laboratory wipe.
9. Place tissue in petri dish and place on scale to weigh. Decide how much to cut for a 10 mg piece per assay reaction. If multiple samples of the same tissue will be assayed, the tissue can be processed in bulk. For example, if 4 reactions of the same tissue will be assayed, all 40 mg of the tissue can be processed in one petri dish.

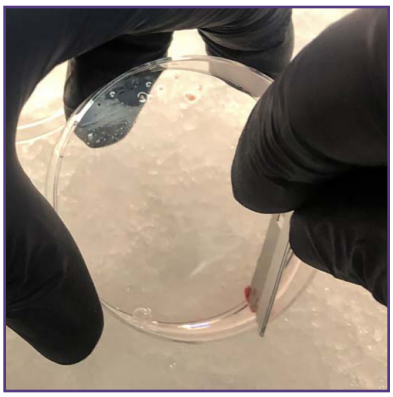
   A. To cut tissue to desired size, place petri dish with tissue back on dry ice.

   B. Hold petri dish firmly and use razor to chop the tissue to smaller pieces. This is to get 10 mg per sample for the reaction. The finer chopping for tissue lysis is in step 12 below.

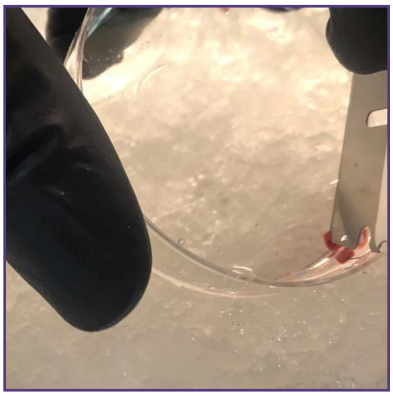


C. Place dish with tissue section on scale to check weight, and repeat adding or dividing sample and chopping until target weight is reached.

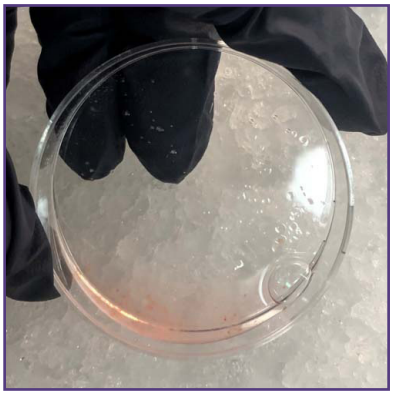
10. Place petri dish with tissue sample on wet ice. Add 1 mL of the freshly prepared Tissue Lysis Buffer - NGS Assays to the 10 mg sample. Lift one side of the petri dish so the tissue and buffer run down to the far side, while the tissue remains covered with buffer. Let sit 1 minute. If processing a larger amount of tissue for multiple reactions, add an adequate amount of Tissue Lysis Buffer - NGS Assays to sufficiently cover the tissue as it is processed.



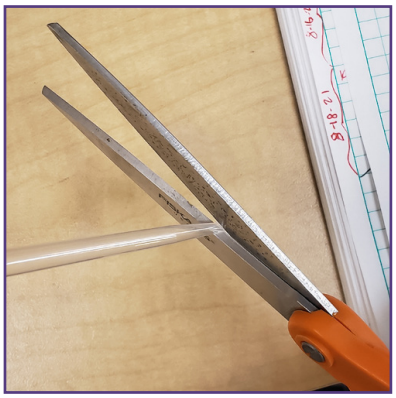
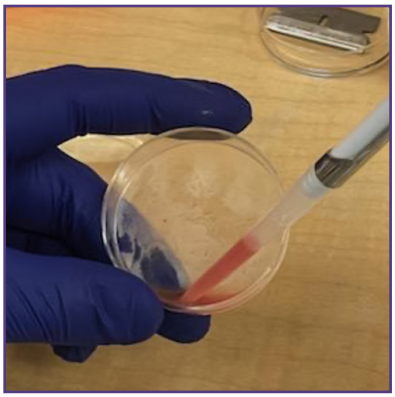
11. Partially lift the petri dish so that the tissue and the buffer run down to the corner of the dish so the tissue stays covered in the Tissue Lysis Buffer - NGS Assays and can be minced into smaller pieces. Using the razor blade, start slicing firmly through the tissue.



12. Lay the petri dish flat and mince all the larger pieces until no chunks of tissue can be seen. Chop until tissue pieces can no longer be chopped into smaller pieces by the razor. Mince until the pieces are small enough that they can be easily aspirated by a P1000 pipette.

13. Using the razor blade, push the finely minced tissue in the Tissue Lysis Buffer - NGS Assays to an edge of the dish. This will make the sample easier to collect.
14. With clean and disinfected scissors, cut the narrow tip off of a 1 mL pipette tip. Use this to pipette the sample out of the petri dish and into the Dounce Homogenizer.

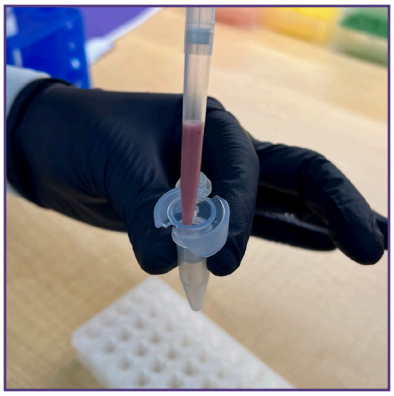



15. Dounce sample with tight pestle 30 times, being careful not to move the pestle above the meniscus to prevent forming bubbles.

    If the pestle is very tight and very difficult to move, first do 20 strokes with the loose pestle, then change to the tighter pestle for 10 strokes.

16. Place a 40 µm Strainer into a microcentrifuge tube on wet ice. One 40 µm Strainer and one corresponding microcentrifuge tube will be needed per 1 mL of sample. These Strainers fit 1.5 ml, 2ml microcentrifuge tubes and 15 ml conical tubes.

17. Pipette the Dounced tissue into the 40 µm Strainer on the microcentrifuge tube. Be careful to avoid large tissue pieces so the mesh does not get clogged. Gently move the 40 µm Strainer up and down inside the tube to help the sample flow through. Hold the tube with one hand and move the Strainer up and down with the other hand without fully removing the Strainer from the tube to create a void for the lysed tissue to flow into. Once the sample has moved through the Strainer, discard the Strainer.



18. Centrifuge sample tubes at 500 X g for 5 minutes at 4°C to pellet nuclei.

19. Remove supernatant from sample and discard.

20. Resuspend pelleted nuclei in 375 µL of ice cold 1 X Wash Buffer per 1.5 ml microcentrifuge tube per sample reaction being assayed. This is the nuclei suspension. If there are multiple reactions of the same sample type, pool them together to count nuclei.

Note: The number of nuclei per mg will vary by tissue type. We observed an average of ~150,000 nuclei per mg. Counting accuracy is critical and we recommend an automated counting method versus a hemocytometer.

21. Take a sample of the nuclei suspension and count. Calculate nuclei density and normalize samples to a concentration of 250,000 nuclei in 375 µL 1 X Wash Buffer per sample. Add freshly prepared 1 X Wash Buffer if needed to obtain that concentration.

Note: If you are normalizing to use the same amount of nuclei per reaction, we recommend CUT&Tag reactions in the range of 100,000 to 500,000 nuclei.

Note: If you have excess nuclei, you may store them in a separate cryo-vial. Add DMSO to 10% of the total volume in the vial. Freeze these samples overnight in an isopropanol freezing container in a -80°C freezer. If you are planning on storing nuclei for more than 1 month, transfer these samples to a liquid nitrogen container.

Section C. Bind Primary Antibody (2 hr to Overnight)

For CUT&Tag R-loop: Take a sample of the nuclei suspension and count. Resuspend sample in 100 µL of 1X Wash Buffer for ConA binding per 500,000 nuclei per sample. Proceed with step to add cells to the freshly prepared Concanavalin A beads in assay protocol.

For CUT&RUN: Take a sample of the nuclei suspension and count. Resuspend sample in 100 µL of 1X Wash Buffer for ConA binding per 500,000 nuclei per sample. Proceed with step to add cells to the freshly prepared Concanavalin A beads in assay protocol.

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

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