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Storage and Processing of Tissue for bulk RNA Isolation

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

Storing Tissue for RNA Isolation

1 After resection, tissue is immediately flash frozen in 50 ml Falcon tube or 2 ml cryovial tubes by placing tissue into tube then simply dropping filled tube into liquid nitrogen.

2 The tubes are stored on dry ice or -80C until they can be stored in a liquid nitrogen tank

Bulk RNA Isolation

3 The large tissue pieces are smashed in a freezing cold mortar and pestle with liquid nitrogen. The desired amount of tissue (30mg - 60mg) is then pulverized and swept into a 1.5 ml centrifuge tube sitting on dry ice to prevent thawing of the tissue.

> Note
>
> Its ciritically important for RNA integrity to not let the tissue thaw. Thawing will activate the endogenous RNases within the tisses thus depleting the chemical we wish to assay.

4 Follow the Quiagen All Prep Kit protocol found here: https://www.qiagen.com/us/resources/resourcedetail?id=bbd50261-3b80-4657-ad58-6a5a97b88821&lang=en. Complete the RNA isolation steps.

5 Breifly, be sure to lyse and homogenize the tissue with RLT buffer with beta-mercaptoethanol with an 18 gauge needle until the needle runs smoothly. Additionally, if once the lysed tissue in RLT appears to be at all yellow and not clear, dilute the lysed tissue in RLT in more RLT buffer. If the RLT is too dense, it will not run through the columns and you'll loose material.

6 Elute in water and freeze at -80C.