RNA Integrity Check V.1

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[1]In-house protocol

Version 1
Nov 23, 2020

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

RNA Integrity Check: Modification of Protocol from Qiagen

PROTOCOL CITATION

Elizabeth Fozo 2020. RNA Integrity Check. **protocols.io** https://protocols.io/view/rna-integrity-check-bpwjmpcn

CREATED

Nov 19, 2020

LAST MODIFIED

Nov 23, 2020

PROTOCOL INTEGER ID

44715

SAFETY WARNINGS

Formamide and formaldehyde are toxic and dangerous chemicals. Handle with care in the hood!

DISCLAIMER:

DISCLAIMER: This is a work in progress.

ABSTRACT

RNA Integrity Check: Modification of Protocol from Qiagen

BEFORE STARTING

Pretreat gel containers and gel flask with RNase Zap

RNA Integrity Check

1 Determine the concentration of RNA; ideally want to run approximately **1 µg** of RNA on the gel (should be about **1 µl** of your sample)

2 Spray gel tray, comb, damns, and a small flask with RNAse Zap.

3 For small gels, melt 0.3 grams of RNA ONLY agarose in 30 ml 1X TBE (or 1X TAE) to the cleaned flask (for a 1% gel using Horizon gel boxes. If using something else, adjust accordingly).

4 Let the solution cool slightly, add **1 µl EtBr** . Pour gel and let solidify.

5 For samples:

1. RNA sample in a total of **8 µl** of water (if RNA concentration is about **1 µg /ml** , add **1 µl** of RNA to **7 µl** of RNAse-free water)
2. **4 µl** of sample buffer
3. Mix samples

Sample Buffer (make FRESH!)

- **80 µl of concentrated dye** (80% glycerol, 0.2% bromophenol blue, 0.2% xylene cyanol; this does NOT need to be made fresh!)
- **500 µl of formamide**
- **120 µl of 37% formaldehyde**

(1/10 of the recipe above is normally enough)

6 Load gel and include a 1 kb DNA ladder for size approximation; run at 100 volts about 45-60 minutes

7 Gel: should see 2 distinct bands corresponding to the 16s and 23s rRNA species. If RNA is intact, will not see smearing. **Should also see lots of tRNA at the bottom**.

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On occasion, I have run a mini-1X MOPS gel to validate RNA integrity especially if I do not have much sample left and really needed to use the RNA for an important experiment.