



RNA Integrity Check V.2

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¹In-house protocol

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Works for me

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Eadewunm

ABSTRACT

RNA Integrity Check: Modification of Protocol from Qiagen

PROTOCOL CITATION

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SAFETY WARNINGS

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

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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  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 $\mu\text{g}/\text{ml}$, 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.