Troubleshooting Forum

Molecular Biology Techniques Q&A

DNase-treated RNA

This month's question from the Molecular Biology Forums (online at molecularbiology.forums.biotechniques.com) comes from the "RNA Methods" section. Entries have been edited for concision and clarity. Mentions of specific products and manufacturers have been retained from the original posts, but do not represent endorsements by, or the opinions of, *BioTechniques*.

Does DNase treatment affect the concentration and purity of isolated RNA? (Thread 31047)

Q I have been using Tri-reagent to simultaneously isolate RNA and protein from mosquito larvae. After isolating RNA using the protocol supplied, I measured the RNA and got an A_{260}/A_{280} ratio of 2.00. But I have been experiencing problems with DNase treatment, which might be degrading my RNA. Following DNase treatment, my ratios either drop to less than 1.6 when I use DNaseI with EDTA and heat inactivation or increase to 2.5 or higher when I use TURBO DNase with the Inactivation reagent. This pattern is consistent. What is going on and how can I resolve it?

A Why do you measure the OD after DNase treatment? Measuring the OD after you complete the RNA purification assesses RNA concentration and purity. You know exactly what components you add for the DNase treatment, so you shouldn't need to check the OD again. If you must know the OD of the RNA following DNase treatment, you need to purify the RNA again to remove the DNase, BSA, and salts in the buffer. Those elements will change the OD readings because they can absorb at A_{280} and A_{230} , shifting the ratios. Degraded pieces of RNA and dNTPs will also absorb at A_{280} .

The change in OD that you are observing does not indicate that your RNA is degraded. Without re-purification of the RNA, reading the OD does not tell you anything.

A The change in your A_{260}/A_{280} ratio was caused by DNase in your RNA sample. Removal of DNA from RNA samples is better done using RNeasy mini spin columns according to the on-filter DNase digestion and RNA cleanup procedure.

Q Actually, I was measuring my samples after LiCl precipitation and washing with 70% ethanol. I thought that should remove the DNase, yet I experienced those problems. In this case, what could be causing these issues? I will try repeating the RNA isolation using other kits, such as RNeasy, RNeasy, and PicoPure RNA isolation. Does anyone have experience with any of these kits? Can you offer any suggestions or warnings?

A I haven't used LiCl precipitation, but if it is similar to ethanol or isopropanol precipitation with acetate or NaCl, it may not remove all contaminants. Proteins such as DNase and BSA may still be present. If you want the RNA to be clean enough for reading the OD, you need to extract and then precipitate. You might try doing this with a spin column if there's an appropriate one available.

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Although purifying the RNA again would allow you to read the OD, I think this introduces more risk to the procedure. If you are concerned about RNA yields after DNase digestion, a housekeeping gene in the PCR will indicate any inequalities in the downstream assay. If you want to minimize variation, just use equal amounts of each RNA diluted to the same concentration for DNase treatment. Then you'll have equal concentrations of DNase digestion components in all the RNA samples and RT-PCR reactions. A dilution series or standard curve of one template will show if the primer set is working efficiently, and the conditions and efficiency should be the same for the other samples. When I do this, I don't worry about the exact composition of the reactions as long as the RT-PCR reactions work well.

A If you zero the NanoDrop with a DNase-treated water sample (e.g. extract a water sample and DNase treat it just as you would an RNA sample) and use that as the blank, you can cancel out the buffer readings at A_{260} and A_{280} . We have done this repeatedly and it has worked well. Trusting that care has been taken to minimize carryover DNA in the RNA samples from the beginning (although some is always there), the absorbance of free dNTPs left over from the DNA chewed up by the DNase will hopefully still be a minimal contribution to the A_{260} .

For TURBO DNase-treated RNA, A_{260}/A_{280} ratios of 2.0 to 2.04 are common for us when used on RNAs from numerous different tissue and cell types isolated through Trizol extraction, DNase-treated, diluted 1:10 with RNaseOUT and nuclease-free water, and then checked with the NanoDrop.

We run the blank side by side with the RNA, so the blanking buffer is actually a complex amalgam of TURBO DNase reagents, RNaseOUT and its buffering elements, and nuclease-free water, along with an amount of water added at the beginning of the extraction process to serve as the stand-in for a biological RNA sample. Too often, people make the mistake of using ddH₂O to initialize and blank the NanoDrop. Rather, you should initialize the NanoDrop with ddH₂O, but you then should zero it using the custom blanking buffer described above.

A You should just read the OD of the RNAs before DNase treatment and keep track of how much they are being diluted by DNase treatment. Then you can just calculate the new concentrations.

A You can use a fluorescent approach such as Qubit with Oligogreen or RiboGreen to

measure ssRNA concentrations. This allows you to measure RNA in the presence of contaminating DNA.

A I have tried the method for generating a blank proposed above. I had one problem, not with nucleic acids, but with protein samples spiked with sodium azide to prevent microbial growth during storage. Sodium azide gives a strong broad absorbance around 280nm, which makes blanking the instrument very difficult. But I have never encountered azide in qPCR sample preps.

A You can use the RNeasy mini kit to clean up the samples you have now. There is no need to start over with new extractions.

Q2 I have also experienced changes in ratios when quantifying RNA samples via Nanodrop after DNase treatment. In my case, it wasn't the A_{260}/A_{280} ratio that was changing, but the A_{260}/A_{230} , which changed from 2 to 1.5. Do you think this would also be solved by using a DNase treated water sample as a blank? I don't understand why the ratios are decreasing. The A_{260}/A_{280} could go down since it would be detecting the DNase, but can anyone offer an explanation for the change in A_{260}/A_{230} ?

A EDTA, carbohydrates, phenol, and guanidine HCl all absorb at 230nm, making the A_{260}/A_{230} ratio smaller than 2.0-2.2. That could be causing your problem. You should use the elution buffer as a blank for the NanoDrop.

A You need a blank containing a solution with the same buffers and other components as the sample to be tested. If the total absorbance is lower, I've found the ratios to be lower. When I started using the Nanodrop and stopped diluting my samples, all the ratios increased toward the ideal number. Following DNase treatment, if there was DNA present, your solution will contain dNTPs that will affect the absorbance. dNTPs absorb at A_{260} .

It is not necessary to worry too much about this unless you are preparing templates for a very expensive or important experiment. After purifying and quantifying nucleic acid, I generally wouldn't try to quantify it again by OD after it is used in a reaction mix unless it was re-purified first. I assume that the RNA is still present, and if I suspect degradation, I check with PCR or by looking for rRNA bands on a gel.

Selected by Kristie Nybo, Ph. D.

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