

Procedure 6.4 – Reagent Preparation – Nuclease-Free Water

Date: _____

Experiment ID: 6.4-*yyyymmdd*

Technician: _____

Project: _____

Client Signature: _____

This procedure is performed to verify nuclease removal from each new lot of water produced by the Elga PureLab Ultra Water Purification System.

1. Prepare the following in micro centrifuge tubes.

Test Sample	19 ul PureLab water 1 ul Total RNA (RIN 10, 5ug/ul)	_____
Test Sample Duplicate	19 ul PureLab water 1 ul Total RNA (RIN 10, 5ug/ul)	_____
Water Control	19 ul Ambion Nuclease-Free Water 1 ul Total RNA (RIN 10, 5ug/ul)	_____
Buffer Control	18 ul Ambion Nuclease-Free Water 1 ul Total RNA (RIN 10, 5ug/ul) 1 ul Rnase One Buffer	_____
Enzyme Treatment	17.5 ul Ambion Nuclease-Free Water 1 ul Total RNA (RIN 10, 5ug/ul) 1 ul Rnase One Buffer 0.5 ul Rnase One	_____

2. Store the Water Control and the original RNA on ice.

3. Incubate all samples except for the Water Control at 37C for two hours.

4. Perform RNA integrity analysis for all samples (BioAnalyzer NanoChip) as described in Appendix 7.2

5. Verify the nuclease activity of the test samples according to the following criteria.

- **The Test Sample, Test Sample Duplicate, Water Control, Buffer Control, and Original RNA should all be completely intact (High RIN).**
- **The Enzyme Treatment should be completely degraded (Low RIN).**

6. Enter the Nuclease-Free water lot into the reagent logbook. _____