Introduction

This Technical Note highlights the improvements of the RNA 6000 Nano and Pico Series II assays with their predecessor assays. The new RNA 6000 Nano and Pico Series II kits will replace the previous RNA kits which will be obsolete by the end of June 2006.

Primary modifications of the RNA 6000 assays are:

- Improved RNA ladder which is now included in both RNA kits
- Improved quantitation accuracy
- Consistently higher sensitivity for the RNA Pico assay
- Quantitation for RNA Pico assay
- Increased assay stability for samples with a high salt content for both RNA assays

Sensitivity

The RNA ladder, which previously had to be ordered separately from Ambion, Inc., is now conveniently integrated into both RNA kits. In addition, it can be ordered separately (table 1). The complete RNA Reagent Kit is shipped at 4 °C, however, the RNA ladder should be aliquoted and stored at -20 °C upon arrival.

<table>
<thead>
<tr>
<th>Series II Products</th>
<th>Order Number</th>
</tr>
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<tbody>
<tr>
<td>RNA 6000 Nano Kit</td>
<td>5067-1511</td>
</tr>
<tr>
<td>RNA 6000 Nano Reagents</td>
<td>5067-1512</td>
</tr>
<tr>
<td>RNA 6000 Nano Ladder</td>
<td>5067-1529</td>
</tr>
<tr>
<td>RNA 6000 Pico Kit</td>
<td>5067-1513</td>
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<td>5067-1514</td>
</tr>
<tr>
<td>RNA 6000 Pico Ladder</td>
<td>5067-1535</td>
</tr>
</tbody>
</table>

Table 1
Ordering Information.
In addition to reagent changes, the assay performance was also improved by major changes to the assay script and firmware. The sensitivity of the Series II RNA 6000 Pico assay was enhanced considerably allowing for the reliable quantitation of as little as 50 pg/µL total RNA in water (figure 2).

The new RNA ladder provides more intense and stable bands (figure 1), especially in the upper size range, which allows for a more reliable integration by the 2100 expert software.

**Sensitivity**

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**Quanitation**

The quantitation calibration has been adjusted to better match data obtained by UV measurements. Together with the more stable RNA ladder this results in a more accurate (figure 3) and robust quantitation (table 2).

**Figure 1**

RNA 6000 ladder. The Series II RNA 6000 Nano ladder was analyzed with the RNA Nano assay (A). The previous ladder was analyzed for comparison (B).

**Figure 2**

Sensitivity: 50 pg/µL total RNA in water was analyzed using the Series II RNA 6000 Pico assay. The data is shown in the normal view (A) and the enhanced RNA view (B), two options provided by the 2100 expert software.
**Salt tolerance**
Compatibility of the assay with different buffers is a critical parameter for the analysis of RNA samples. In particular, tolerance to different salts is important because salt can impact assay sensitivity. A modification of both RNA assay scripts increased the sample salt tolerance significantly, for example for the Pico assay from 10 mM Tris, 0.1 mM EDTA to 50 mM Tris, and 0.1 mM EDTA (figure 4). The reproducibility of the injection of RNA samples in such sample buffers is improved especially for the 28S ribosomal RNA. This is reflected in the ribosomal RNA ratio, RIN and in the quantitation. In general, the signal intensity and quantitation reproducibility decreases with increasing salt concentrations in the sample buffer.

**Further improvements**
Further improvements include:
- The vortexing speed was decreased to 2000 rpm to avoid liquid spilling of RNA samples containing detergents. This eliminates the risk of leak current between the sample wells.
- Both RNA 6000 kits now include 2.4 mL of marker solution. The increased volume ensures that the amount of marker solution is sufficient also for usage as a sample in blank runs.
- The lifetime of the RNA 6000 Nano and Pico reagents is initially extended to a minimum of four months after shipment, if stored appropriately. Additional tests are in progress to further extend the lifetime.

With the above described modifications, the Series II RNA 6000 assays provide an improvement in specifications over the previous RNA assays (table 3).
Figure 4
Improved salt tolerance. Total RNA in 10 mM Tris, 100 mM Tris and 50 mM NaCl was analyzed using the previous RNA Pico assay (A) and the new Series II RNA 6000 Pico assay (B).

Table 3
Series II RNA 6000 specifications. The specifications of the previous and the new RNA assays are compared.

* ladder as sample