Institute for Systems Biology – Alexa Dye Labeling Protocol

***Do not use the protocol that comes with the kit, it doesn't work as well.**

***Do not precipitate RNA or DNA to be labeled with ammonium acetate! It reacts with the platinum catalyst.**

- Label RNA or sonicated/digested DNA in **100% DMF**
- Add dye (see chart) to the RNA/DNA and bring to a total volume of **20 ul**.

	594	660
10ug Total RNA	6ul	12ul
2ug cRNA or mRNA	2ul	4ul

- Mix dye and RNA/DNA and put on 95 degree heat block for 5 minutes. Put directly in ice slurry afterward to stop reaction.
- Precipitate at room temp with 2 ul Ambion glycogen (5ug/ul), 0.4 ul LiCl (8M), and 25 ul of 100% isopropanol. Mix and let sit for ten minutes.
- Spin at 4000g for 10 minutes.
- You should see a **big bright pellet,** if not try cooling sample and spin again. Dump isopropanol.
- Wash with 100 ul 70 % ethanol.
- Spin at 4000g for 5 minutes. Dump ethanol.
- Dry pellet and resuspend in 60 ul DEPC water.

You will have to put your whole reaction on the spec to get the reading for your dye absorbance. Dissolving 2 ul in a total of 50 ul of water is usually a good dilution for getting your RNA/DNA concentration.

You can also use Biorad Biospin 30 columns to clean up your reactions and skip the isopropanol precipitation but they are expensive.