Radiolabelled 1-nt RNA ladder

AIM: Generate a 1-nt radiolabelled RNA ladder that can be used to calculate the size of small RNA products on gel. This is based on alkaline degradation of the radiolabelled RNA which results in a 1nt resolution ladder.

Materials:

- 5'end-labelled RNA oligo of your choice (see "End-labelling with T4 PNK" protocol)
- 65°C bath or block heater
- 37°C bath or block heater
- Ice bucket
- NaOH 2N solution
- Tris 1M pH7,5
- RNA dye

Solutions:

RNA dye	10ml
94% Formamide	8.8ml of 100%
0.05% Bromophenol blue	1ml of 1%
0.05% Xylene cyanol	200ul of 5%

Store at RT

Method:

1. Mix:
    - 1,5ul 5'-end labelled RNA
    - 1ul DEPC-water
2. Incubate 1min at 65°C.
3. Quickly put on ice. Incubate 2min on ice.
4. Incubate 3min at 37°C.
5. Add 1ul NaOH 2N and incubate 2min at room temp.
6. Stop reaction with 2ul Tris 1M
7. Add 5ul RNA dye
8. Store at -20°C

For loading, a 1/50 dilution in RNA dye is usually ok, but can vary according to your experiment. Adjust dilution if necessary.