Quant-IT RiboGreen RNA QUANTIFICATION FOR LOW RANGE ASSAY (1ng/ml~50ng/ml)

Kit used: Quant-iT™ RiboGreen® RNA Assay Kit, ca. #R11490

Standard Curve Preparation:

1. Prepare 1×TE buffer (20× fold diluted in PCR water):
   Mix 100 µL of Component B (20×TE buffer) with 1900 µL of PCR water to make 2000 µL of 1×TE buffer

2. Prepare 100ng/mL RNA stock (1000× fold diluted in 1×TE buffer):
   Mix 1 µL of component C (100 µg/mL RNA standard) with 9 µL of 1× TE buffer to make 10 µL of 10 µg/mL RNA stock. And then mix 2 µL of 10 µg/mL RNA stock with 198 µL 1×TE buffer to make 200 µL of 100ng/mL RNA stock.

3. Prepare RiboGreen solution (2000× fold diluted in 1× TE buffer):
   Mix 1 µL of component A (Quant-IT RiboGreen RNA reagent) with 1999 µL 1× TE buffer to make 2000 µL RiboGreen working solution.

4. Prepare a low-range standard curve

1×TE buffer (µL)	100ng/mL RNA stock (µL)	2000× diluted RiboGreen solution (µL)	Final RNA concentration in Assay
0	100	100	50ng/mL
50	50	100	25ng/mL
90	10	100	5ng/mL
98	2	100	1ng/mL
100	0	100	blank
85~90	Sample 10~15 µL	100	sample

5. Before measurement, add 100 µL of RiboGreen solution to tube and incubate 5 min at RT protected from light.

6. Measure the signal in the spectrofluorometer (Nanodrop 3300). Load 2 µL for each measure and 3 independent measurements for each concentration to reduce the reading variation due to low concentration.