Product Data Sheet

Product Name: RiboGreen RNA Reagent
Cat. No.: GC26545

Chemical Properties

Cas. No.			
Formula		M.Wt	
Solubility		Storage	Store at 4°C,protect from light
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Protocol

1. Overview

In RiboGreen's RNA quantification detection, two different dye concentrations are required to obtain a complete linear kinetic range. Before preparing the RiboGreen working solution (see reagent preparation below), it is necessary to decide whether to perform a high range assay (20ng/mL-500ng/mL RNA), a low range assay (1ng/mL-50ng/mL RNA), or both ranges.

2. Preparation of reagents

On the day of the experiment, RiboGreen's water-soluble working solution was prepared in a sterile disposable polypropylene plastic tube. Avoid using glassware as RiboGreen may adhere to glass surfaces. The prepared RiboGreen working solution should be stored away from light. It is best to use up the working fluid within a few hours after preparation, and do not store it for long-term use.This product is supplied as a stock solution dissolved in DMSO, with a concentration of 200x (High-range

assay) and 2000x (Low-range assay). Based on a 2ml reaction system (cuvette), a 100μL sample can perform 20 reactions (High-range assay) and a 100μL sample can perform 200 reactions (Low-range assay).

3. Prepare RNA standard curve

For the standard curve, commercial RNA standards can be used, such as the commonly used 16S and 23S ribosomal RNA; Or other purified RNA products. Sometimes, it is recommended to use RNA standards similar to the sample being tested to establish a standard curve. Generally speaking, the vast majority of single stranded RNA molecules can produce almost equivalent fluorescence signals. In the presence of common compounds that contaminate nucleic acid reagents such as nucleosides, salts, urea, ethanol, chloroform, detergents, proteins, and agarose, RiboGreen's detection results can still maintain a good linear relationship, even if the fluorescence intensity may be affected . In order to ensure effective control, the RNA solution used to prepare the standard curve should be consistent with the sample to be tested, which can ensure the presence of impurity compounds at similar levels.

3.1 Prepare a 2 μ g/mL RNA solution in a plastic bottle using a 1xTE buffer. Measure the basic absorbance of the RNA solution at 260nm (A260) using a colorimetric cup in a 1cm long pathway. A260=0.05 is equivalent to a concentration of 2 μ g/mL of RNA A.

When preparing the High range standard curve, a series of 2x final concentration RNA standard solutions are prepared by diluting 2 μ g/mL RNA solution into a disposable nuclease free plastic tube, and finally transferred to a colorimetric dish or culture plate.

3.3 When preparing the Low Range standard curve, a series of 2x final concentration RNA standard solutions are prepared by diluting 2 μ g/mL RNA solution into a disposable nuclease free plastic tube, and finally transferred to a colorimetric dish or culture plate

3.4 Mix equal volumes of RiboGreen reagent working solution (see 2-Reagent Preparation) and 2xRNA standard solution. Incubate at room temperature in the dark for 2-5 minutes, ensuring that a sufficient volume of measuring solution is added to the measuring container. For a 10x10mm colorimetric dish, it should not be less than 2mL; For each hole of the 96 well plate, do not lower it than 200 μ L.

3.5 Choose to use a fluorescence spectrophotometer or fluorescence enzyme-linked immunosorbent assay (ELISA) reader to measure the fluorescence value of the sample, and select the standard fluorescence wavelength (excitation~480nm, emission~520nm).

3.6 Subtract the blank fluorescence value from the fluorescence value of each sample. Afterwards, use the calibrated fluorescence values as the y-axis and RNA concentration as the x-axis to establish a standard curve.

4. Sample analysis

Product Data Sheet

4.1 Dilute the sample with a 1xTE buffer to an appropriate volume (e.g. 1.0mL to 10x10 mm cuvette or 100 μ L to 96 well microplate). It is best to do more than one dilution factor for each sample to be tested.
The higher the dilution factor of the test sample, the more helpful it is to reduce the interference of specific pollutants. However, it is also necessary to avoid particularly small sample volumes as they are difficult to measure accurately. In addition, in the same experiment, the impurity levels should be kept as consistent as possible to reduce the signal differences between samples caused by impurities. For example, if a series of RNA samples have significantly different salt concentrations, they cannot be compared using a simple standard curve. To avoid such problems, if possible, adjust the impurity concentration in all samples to the same level. (See 5- DNA clearance in sample)
4.2 Add an equal volume of RiboGreen measuring reagent (see 2-Reagent Preparation) to each sample and incubate at room temperature in the dark for 2-5 minutes.
4.3 Select the same instrument parameters as the standard curve measurement to determine the fluorescence value of the sample. In order to reduce the photobleaching effect, the fluorescence measurement time of all samples remains unchanged.
4.4 Subtract the blank fluorescence value from the fluorescence value of each sample. Determine the RNA concentration of the sample based on the generated RNA standard curve.
If possible, verify the quantitative results by diluting different samples and repeating the measurements.
5. DNA clearance in the sample
RiboGreen can also bind to DNA, and RNA free DNases can be used to remove DNA from the sample first, thereby avoiding fluorescence interference caused by the binding of DNA and RiboGreen, ensuring that all fluorescence in the sample comes from the binding of RiboGreen and RNA.
5.1 Prepare 10x DNase digestion buffer: 200mM Tris HCl without nuclease, pH 7.5,100 mM $MgCl_2$,20 mM $CaCl_2$。
5.2 Add 0.11 times the volume of 10xDNase digestion buffer to each DNA containing sample. (For example, if the sample is 9mL, add 1mL of 10x buffer).
5.3 Add~5 units of DNase without RNase to 1 μ g of DNA.
5.4 Incubate at 37 ° C for 90 minutes.
5.5 Dilute the sample at least 10 times with a 1xTE buffer to reduce interference from residual salt ions in the digestion solution on RiboGreen measurements.
5.6 Perform RiboGreen assay experiment according to the above steps.

Product Data Sheet

Background

RiboGreen RNA Reagent is an ultra sensitive fluorescent nucleic acid dye used for quantitative detection of RNA in solution. The detection and quantification of trace RNA are particularly important in a large number of molecular biology experiments, including the determination of in vitro transcription RNA production, as well as the determination of RNA concentration before conducting Nor Thern Blot, S1 nuclease experiments, RNase protection experiments, cDNA library preparation, reverse transcription PCR, and differential display PCR.

The most common method for nucleic acid concentration determination is to measure the absorbance value at a wavelength of 260nm (A260). However, the main drawback of the absorbance based detection method is that proteins and free nucleotides have significant interference with the light signal; And the detection sensitivity is low (A260=0.1 is equivalent to 4 μ g/mL RNA in solution); The application of sensitive fluorescent nucleic acid dyes can avoid many problems caused by these factors.

RiboGreen can quantify RNA at concentrations as low as 1ng/mL and measure it using a fluorescence enzyme-linked immunosorbent assay (ELISA) reader, standard fluorescence spectrophotometer, or filter fluorometer. The maximum excitation wavelength of this probe after binding to RNA is around 500nm, and the maximum emission wavelength is around 525nm. Reading with a fluorescence enzyme-linked immunosorbent assay (ELISA) reader, a reaction system of 200 μ L can detect RNA as low as 200 pg, which is 200 times more sensitive than the fluorescence analysis method based on ethidium bromide and 1000 times more sensitive than the analysis method based on ultraviolet absorption peak. Using two dye concentrations, RiboGreen can measure RNA concentrations within a linear range of up to three orders of magnitude, ranging from 1ng/mL to 1 μ g/mL RNA. High range assay can quantify RNA ranging from 20ng/mL to 500ng/mL, while low range assay can quantify RNA ranging from 1ng/mL to 50ng/mL. Even in the presence of several common impurities in nucleic acid preparations (including nucleotides, salts, urea, ethanol, chloroform, surfactants, proteins, and agarose) in the system, this linear relationship can be maintained. In addition, RiboGreen can also bind to DNA by pre treating the mixed sample with DNase and then quantitatively analyzing RNA selectivity using RiboGreen.

Caution: Product has not been fully validated for medical applications. For research use only.
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