

Standard Operating Procedure for Quantitation of DNA and RNA

Medical University Innsbruck
Internal Medicine IV
Nephrology and Hypertension

Purpose

This protocol describes the required procedure for the quantitation of RNA and DNA from kidney biopsies using PicoGreen and RiboGreen Reagent Kits (Molecular Probes)

Scope

This procedural format is utilized by the contributing centres to the project 'Functional Significance of Changes in Genome Wide Gene Expression Profiles Associated with Renal Aging'. Adherence to the protocol will standardize the quantitation of RNA and DNA extracted from the human kidney biopsy samples collected for this project.

Materials

Quant-iT RiboGreen RNA Assay Kit (Molecular Probes)
PicoGreen dsDNA Quantitation Kit (Molecular Probes).
96-well clear flat-bottom Microplates
RNase-free microfuge tubes and pipette tips

Procedure

The following procedure is adapted from the Molecular Probes Protocol, Jan 2001.

RiboGreen RNA Quantitation

Prepare standards

Dilute 15 ul of ribosomal RNA standard (Component C – 100 ug / ml) in 985 ul 1x TE buffer. This solution contains 1500 ng / ml RNA and will be the top standard (St1) for the standard curve. For St2 add 300 ul of St1 to 600 ul of 1x TE buffer, briefly vortex and zip spin. St2 contains 500 ng / ml RNA. Continue diluting 300 ul of each consecutive standard 600 ul 1x TE buffer until St7 which will contain 2 ng / ml RNA. These standards can be frozen at -20°C and re-used across multiple plates.

Prepare RNA samples

Dilute 1 ul of RNA in 44 ul 1x TE buffer (1 in 45 dilution), vortex briefly and zip spin. If the quantity of RNA is expected to be greater than 2 ug, prepare a more dilute sample as well.

Prepare RiboGreen Reagent

The RiboGreen stock solution (Component A) must be diluted in 1x TE buffer before use at a ratio of 1:3200. A volume of 180 ul is required for each well, if using an entire 96 well plate prepare 20 ml of reagent by adding 6.4 ul Component A to 20 ml 1x TE buffer. This solution is light sensitive; keep the solution in the dark and use it as soon as possible.

Loading the 96 well plate

Add 20ul of each standard and sample to duplicate wells - example template below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	1500	1500	1	1	9	9	1	1	9	9		
B	500	500	2	2	10	10	2	2	10	10		
C	167	167	3	3	11	11	3	3	11	11		
D	56	56	4	4	12	12	4	4	12	12		
E	18.7	18.7	5	5	13	13	5	5	13	13		
F	6.2	6.2	6	6	14	14	6	6	14	14		
G	2	2	7	7	15	15	7	7	15	15		
H	0	0	8	8	Blank	Blank	8	8	Blank	Blank		
	standards		Dil. 1/45		Dil. 1/45		Dil. 1/200		Dil. 1/200			

To each well containing sample or standard add 180 ul of RiboGreen Reagent. Protect plate from light and measure fluorescence using a plate-reader as soon as possible.

PicoGreen DNA Quantitation

Prepare Standards

Dilute 2.5 ul lambda DNA standard (Component C – 100 ug/ml) in 247.5 ul 1 x TE buffer. This solution contains 1ug/ml DNA and will be the top standard (St1) for the standard curve, when diluted with PicoGreen reagent this St1 = 500 ng/ml. For standard 2 dilute 120 ul of St1 with 120 ul 1 x TE buffer, briefly vortex and zip spin. Following addition of PicoGreen reagent St2 = 250 ng/ml DNA. Continue diluting 120 ul of each consecutive standard with 120 ul 1 x TE buffer until St7, which will contain 7.8 ng/ml DNA.

Prepare DNA samples

Dilute 3 ul of DNA in 102 ul 1 x TE buffer (1 in 35 dilution), vortex briefly and zip spin. If the DNA yield is expected to be greater than 1 ug, prepare a more dilute sample to measure as well.

Prepare the PicoGreen Reagent

The PicoGreen dsDNA quantitation reagent (Component A) must be diluted in 1x TE buffer before use at a ratio of 1:200. A volume of 50 ul is required for each well, if using an entire 96 well plate prepare 5 ml of reagent by adding 25 ul Component A to 4.975 ml 1x TE buffer. This solution is light sensitive; keep the solution in the dark and use it as soon as possible.

Loading the 96 well plate

Add 50 ul of each sample and standard to duplicate wells. Add 50 ul of the diluted PicoGreen to each well and wrap plate in foil to protect it from light. Incubate plate for 5 minutes at room temperature. Keep plate cool and measure fluorescence using a plate-reader as soon as possible.

Using the Tecan platereader

TECAN GENIUS PLUS (RFU-reading):

Software: XFluor4 button on tool bar

XFluor4 → connect

Select instrument: Genius Plus

XFluor4 → move plate an filter: in/out

XFluor4 → edit measurement parameters:

General: ● Fluorescence

Plate: Part of the plate + rows, GRE96fb/GRE96fc.pdf (black or clear plate)

Measure Parameters: Fluorescein (485-535);

Gain: optimal;

Read Mode: Bottom (must be clear!)

XFluor4 → Start measurement