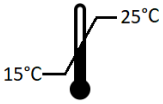
Product Manual

GreenQuant dsDNA Kit

REF NAGS299,1000 – NAGS299,0025

Cyanagen srl, Via Andrea Costa 4/2- 40134 Bologna, Italy



Store at +4°C

September 2023/Rev 01

www.cyanagen.com

About us

Cyanagen is a biotech company located in Bologna, dedicated to research, development, and production of reagents for life science since 2003 and one of the leading companies in reagents for Western blotting and ELISA. Since 2020 the company entered the IVD market with nucleic acids extraction kits based on proprietary magnetic beads.
The main product lines are focused on chemiluminescent and fluorescent substrates and nucleic acids extraction kits for biological analysis, genomics, proteomics and molecular diagnostics. Most of Cyanagen products are based on Cyanagen's internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.
Customer satisfaction and product top quality are of paramount importance to us.

Cyanagen Srl has a certified Quality System

ISO 9001:2015 QUALITY CERTIFIED

ISO 13485:2016 QUALITY CERTIFIED

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1. General information

1.1. Description

The GreenQuant dsDNA Kit contains a fluorescent proprietary DNA-binding dye (488nmEx/522nmEm, **Fig.1**) that allows accurate and sensitive quantitation of small amounts of double-stranded DNA (dsDNA) in a purified sample.

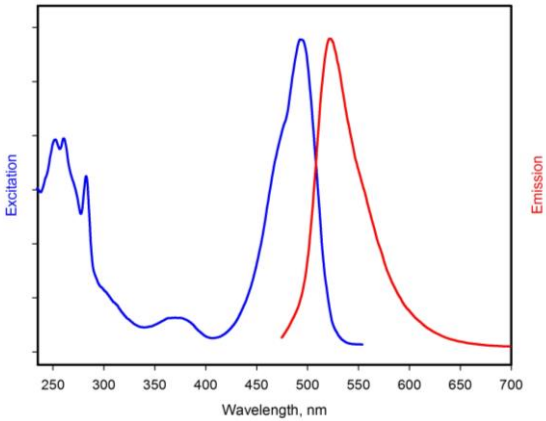

Fig.1 Abs/Em spectra of GreenQuant Dye bound to dsDNA.

The assay is highly selective for dsDNA over RNA (**Fig. 2**) and provides a linear range between 0.2 and 200 ng of dsDNA input, which corresponds to sample concentrations of 0.1 ng/µL to 100 ng/µL. For more concentrated DNA samples, it is recommended to dilute the samples with GreenQuant Buffer 1X. Compared to spectrophotometric DNA quantitation, the assay provides key advantages such as significantly increased sensitivity, high selectivity for double-stranded DNA (dsDNA) over single-stranded DNA (ssDNA) or RNA, and improved contaminant tolerance (protein and carbohydrate molecules). The kit is ideal for quantifying DNA from low-concentration or precious samples, and for quantifying DNA for use in sensitive applications such as Next-Generation Sequencing (NGS) or digital PCR.

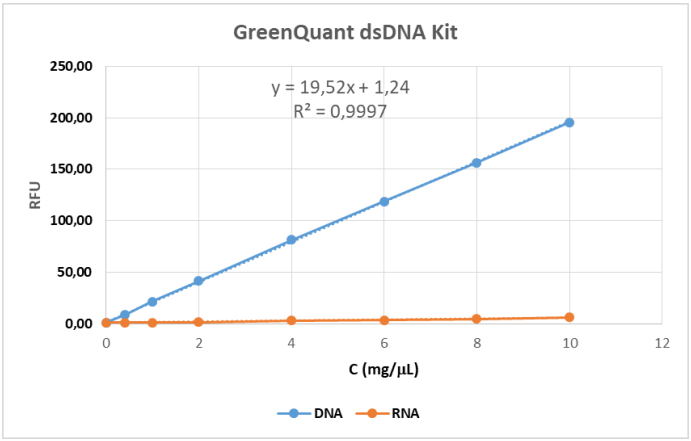

Fig.2 High selectivity of GreenQuant dsDNA Kit for dsDNA over RNA. 10 µL samples of dsDNA and RNA from yeast were assayed in triplicate with GreenQuant dsDNA kit. Fluorescence was plotted against the concentration of the RNA or DNA sample. Background fluorescence had not been subtracted and the y-intercept was set at the RFU of the blank.

The GreenQuant dsDNA Kit includes concentrated GreenQuant Dye, dilution buffer 100X, and DNA λ 20 ng/µL to prepare serial dilutions for standard curve. The kit is intended to be used in combination with any single tube fluorometer and any fluorescence microplate reader at the appropriate excitation and emission wavelengths. The signal is stable for up to 4-6 hours when the samples are protected from light.

The kit is sufficient for 25 or 1000 assays.

2. Components and other required materials

2.1. Kit size and content

The Kit is available in 2 sizes:

NAGS299,1000 (1000 assays)
NAGS299,0025 (25 assays)

The kits include:

Components	GHS	Storage	NAGS299,1000: Ref (Amount)	NAGS299,0025: Ref (Amount)
GreenQuant Dye	**/**	**2-8° C**	**NAGS295,0005 (2 x 0.5 mL)**	**NAGS295,000025 (1 x 25 µL)**
GreenQuant Buffer 100X	**/**	**2-8° C**	**NAGS296,0015 (2 x 1.5 mL)**	**NAGS296,0015 (1 x 1.5 mL)**
GreenQuant DNA λ	**/**	**2-8° C**	**NAGS297,001 (1 x 1 mL)**	**NAGS297,0002 (1 x 200 µL)**

Note. Please note that components from different batches cannot be used interchangeably.

2.2. Shipping and storage

The kit is shipped refrigerated and must be stored at 2-8° C.

2.3. Required materials to be supplied by the user

2.3.1. Equipment and consumable

- Micropipettes suitable for pipetting 10-20 µL, 150 µL, 300 µL, 500 µL
- Vortex
- DNase / RNase-free tubes or plates
- 100 mL amber bottle
- 1,5 mL tubes
- Fluorescence microplate reader or single tube fluorometer with the dedicated tubes/plates

2.3.2. Reagents

- DNase/RNase-free water

3. Before starting (Kit NAGS299,1000)

Prepare GreenQuant Buffer 1X, GreenQuant WS and DNA standards according to paragraph **3.1**, **3.2** and **3.3**.

3.1. GreenQuant Buffer 1X

Add 1 mL of **GreenQuant Buffer 100X** to 99 mL of DNase/RNase-free water in a dark 100 mL bottle (not provided) and mix.

3.2. GreenQuant WS (Working Solution)

GreenQuant Dye is supplied as concentrated**.** The kit contains 2 vials of GreenQuant Dye, allowing to prepare 2 x 100 mL **GreenQuant WS**, which are stable for 6 months at +4°C after the date of reconstitution. Prepare **GreenQuant WS** by transferring all the content of each vial of GreenQuant Dye in the relative **GreenQuant Buffer 1X** bottle prepared in step **3.1**.
Store at +4°C.

3.3. GreenQuant DNA Standards

GreenQuant DNA λ contains 1 mL of Lambda DNA at 20 ng/µL concentration.

- Label eight 1.5mL tubes for each standard as follows: 100, 80, 60, 40, 20, 10, 4, 0.
- Prepare 10 mL **GreenQuant Buffer 1X** by diluting 100 µL of **GreenQuant Buffer 100X** with DNase/RNase-free water up to 10 mL.
- Prepare dilutions of **GreenQuant DNA** λ according to the following table:

Table of DNA standard dilutions

Green Quant DNA Standard (ng/well)	Conc (ng/µL)	GreenQuant DNA λ (µL)	GreenQuant Buffer 1X (µL)
100	10	250	250
80	8	200	300
60	6	150	350
40	4	100	400
20	2	50	450
10	1	25	475
4	0,4	10	490
0	0	0	500

Store the standards at +4°C.

3.4. GreenQuant DNA Standards for use with Qubit™ instruments (Thermofisher)

GreenQuant DNA λ contains 1 mL of Lambda DNA at 20 ng/μL concentration.

- Label two 1.5mL tubes for each standard as follows: Standard 1 and Standard 2.
- Prepare 10 mL **GreenQuant Buffer 1X** by diluting 100 μL of **GreenQuant Buffer 100X** with DNase/RNase-free water up to 10 mL.
- Prepare dilutions of **GreenQuant DNA λ** according to the following table:

Table of DNA standard dilutions			
Green Quant DNA Standard	Conc (ng/μL)	GreenQuant DNA λ (μL)	GreenQuant Buffer 1X (μL)
1	0	0	1000
2	10	500	500

Store the standards at +4°C.

4. Before starting (Kit NAGS299,0025)

Prepare GreenQuant Buffer 1X, GreenQuant WS and DNA standards according to paragraph **4.1, 4.2 and 4.3.**

4.1. GreenQuant Buffer 1X

Add 50 µL of **GreenQuant Buffer 100X** to 4,95 mL of DNase/RNase-free water in a 15 mL falcon tube (not provided) and mix.

4.2. GreenQuant WS (Working Solution)

GreenQuant Dye is supplied as concentrated**.** The kit contain 1 vial of GreenQuant Dye, allowing to prepare 5 mL of **GreenQuant WS**, that is stable for 6 months at +4°C after the date of reconstitution. Prepare **GreenQuant WS** by transferring all the content of the vial of GreenQuant Dye in the relative **GreenQuant Buffer 1X** falcon prepared in step **4.1**.
Store at +4°C and protect from light.

4.3. GreenQuant DNA Standards

GreenQuant DNA λ contains 0,2 mL of Lambda DNA at 20 ng/µL concentration.

- Label eight 1.5mL tubes for each standard as follows: 100, 80, 60, 40, 20, 10, 4, 0.
- Prepare 10 mL **GreenQuant Buffer 1X** by diluting 100 µL of **GreenQuant Buffer 100X** with DNase/RNase-free water up to 10 mL.
- Prepare dilutions of **GreenQuant DNA λ** according to the following table:

Table of DNA standard dilutions

Green Quant DNA Standard (ng/well)	Conc (ng/µL)	GreenQuant DNA λ (µL)	GreenQuant Buffer 1X (µL)
100	10	50	50
80	8	40	60
60	6	30	70
40	4	20	80
20	2	10	90
10	1	5	95
4	0,4	2	98
0	0	0	100

Store the standards at +4°C.

4.4. GreenQuant DNA Standards for use with Qubit™ instruments (Thermofisher)

GreenQuant DNA λ contains 200 µL of Lambda DNA at 20 ng/µL concentration.

- Label two 1.5 mL tubes for each standard as follows: Standard 1 and Standard 2.
- Prepare 10 mL **GreenQuant Buffer 1X** by diluting 100 µL of **GreenQuant Buffer 100X** with DNase/RNase-free water up to 10 mL.
- Prepare dilutions of **GreenQuant DNA λ** according to the following table:

Table of DNA standard dilutions			
Green Quant DNA Standard	**Conc (ng/µL)**	**GreenQuant DNA λ (µL)**	**GreenQuant Buffer 1X (µL)**
1	**0**	0	100
2	**10**	50	50

Store the standards at +4°C.

5. Protocol for the quantification of dsDNA samples using single tube fluorometer or a microplate reader

- Allow all the reagents to warm up at room temperature.
- Add 10 µL of each **GreenQuant DNA standard** prepared in **3.3** to separate tubes or wells of a 96 well plate. At least two replicates for each sample are recommended.
- Add 2 µL of each **unknown DNA sample** to separate tubes or wells of a 96 well plate. At least two replicates for each unknown DNA sample are recommended.
  Note: Sample volume up to 10 µL for each unknown DNA sample can be used.
- Add 200 µL of the **GreenQuant WS** into each tube or well of the 96 well plate containing standards and unknown samples and mix well by pipetting up and down.
- Mix the tubes or the 96 well plate using a plate shaker for about 3-10 seconds.
- After mixing, allow the tubes or the 96 well plate to incubate at room temperature for 5 minutes. The tubes or the plate can be stored in the dark for up to 4-6 hours at ambient temperature before reading.
- Measure fluorescence using a single tube fluorometer or a microplate reader set to 488 nm excitation/522 nm emission maxima or other filter combination for detecting green fluorescence (e.g., FITC filter~480/530 nm). The fluorescence signal is stable for 4-6 hours at room temperature when protected from light.
- To determine the unknown DNA concentration, generate a standard curve. Average the values for each standard sample. Plot the fluorescence values for the GreenQuant DNA standards on the y-axis (RLU) and the amount of DNA in the tube or well on the x-axis (ng/well) and fit a trend line through these points to generate a standard curve with linear equation. For best results, manually set the y-intercept as the RFU value obtained from the 0 ng dsDNA standard.

$$y = \mathbf{a}x + \mathbf{b}$$

- Calculate the coefficient of determination R^2. When properly used, the kit provides R^2 >0,99.

- Use the equation for the standard curve trend line to calculate the amount of unknown DNA in each tube or well (ng/well):

$$x = (y-\mathbf{b})/\mathbf{a}$$

- Calculate the concentration of the unknown sample by dividing it by 2 or by the amount (µL) of samples used.

6. Protocol for the quantification of dsDNA samples using Qubit™ instruments (Thermofisher)

- Allow all the reagents to warm up at room temperature.
- Add 10 µL of each **GreenQuant DNA standard** prepared in **3.4** e/o **4.4** to the appropriate tube.
- Add 1-20 µL of each sample to the appropriate tube.
- Add the **GreenQuant WS** to each tube to get a final volume of 200 µL.
- Gently vortex or invert the tubes several times to mix.
- Allow the tubes to incubate at room temperature for 2 minutes.
- Turn on the Qubit™ instruments (Thermofisher)
- Proceed to "Read standards and samples". Follow the procedure appropriate for your instrument.

5. Troubleshooting

Problem	Possible Cause and comments
Low or no signal in the standard samples	Standard samples not correctly diluted. Verify the concentration of standards by Nano Spectrophotometer
	The GreenQuant WS was exposed to light. Exposure to light will reduce the sensitivity of the assay. Store GreenQuant Dye and working solution protected from light.
Too high signal in the standard samples	Standard samples not correctly diluted. Verify the concentration of standards by Nano Spectrophotometer
	Reader setting not appropriate. Adjust reader parameters on your fluorometer to lower the signal so that the highest point on the standard curve is approximately 90% of maximum signal
Nonlinear standard curve	Standard samples not correctly diluted. Verify the concentration of standards by Nano Spectrophotometer. If necessary, prepare new standards
	Reader setting not appropriate. Adjust reader parameters on your fluorometer to lower the signal so that the highest point on the standard curve is approximately 90% of maximum signal
	A concentration gradient may have formed if the GreenQuant DNA λ arrived frozen. Store the GreenQuant DNA λ at 2-8°C overnight, then warm to room temperature and mix well before use. Do not refreeze the GreenQuant DNA λ.

6. Warning and Precautions

- When working with chemicals, always wear protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.). For more information, please refer to the appropriate material safety data sheets (MSDSs) available online at www.cyanagen.com\MSDS\
- Clinical samples and other specimens to be tested should be considered as potentially infectious substances and processed strictly according to laboratory biosafety requirements.
- Components from different batches cannot be used interchangeably. Do not collect reagents from other bottles of the same lot. After use, immediately close all bottles to avoid leakage, changes in buffer concentrations or buffer contamination. After the first opening, keep all the bottles in an upright position.
- Do not use a kit after the expiration date.
- Avoid any nuclease contamination. Always wear gloves and change them often, especially after contact with skin, hair or other potentially nuclease-contaminated surfaces. Use nuclease-free solutions and nuclease- free certified, disposable plastic ware and filter tips. Maintain a separate area for nucleic acids work. Carefully clean all surfaces.
- In case of spillage or damage to the bottles, dispose of the components as chemical waste according to local safety regulations.
- Should a user detect the Product's malfunction concerning the stated specifications, download the claim form at https://www.CYANAGEN.com/cyanacontent/uploads/Pages-content/Support/support-request-form1.pdf, fill and submit it to CYANAGEN, technical.support@CYANAGEN.com, for internal quality analysis.

Contact and technical support information:
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7. Ordering information

PRODUCT	ORDER - NO	UNIT SIZE
GreenQuant ds DNA Kit	NAGS299,1000	Up to 1000 rxn
GreenQuant ds DNA Kit, trial kit	NAGS299,0025	Up to 25 rxn

For further information

visit **www.cyanagen.com**

contact **technical.support@cyanagen.com**

For orders: **sales@cyanagen.com**

Warranty Disclaimer at
www.cyanagen.com/warranty-disclaimer/


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