MANUAL

RayBio® Microplate dsDNA Quantification Assay

Catalog #: FQA-DS-48, FQA-DS-96, FQA-DS-192

RayBiotech Empowering your proteomics

ISO 13485:2016

Purpose

The RayBio® Microplate dsDNA Quantification Assay is a rapid, sensitive, and simple method to measure the total amount of double stranded DNA in solution using a standard microplate reader. This assay is highly selective for double stranded DNA over single stranded DNA or RNA (Figure 2) and is resistant to interference from common contaminants (Table 1). The range of this kit is from 0.25 ng to 50 ng (5 pg/µL to 50 ng/µL sample concentration).

Kit Components

Store the kit at 4ºC protected from light for up to 12 months. Kit must be used within expiry period to ensure optimal performance. Do not mix reagents from different kit lots.

COMPONENT	CATALOG NUMBER	48 TESTS	96 TESTS	2 x 96 TESTS
DNA Reference, 1 ng/µL	FQA-DS-POS	1 x 500 µL vial		2 x 500 µL vials
Negative Reference	FQA-NEG	1 x 500 µL vial		2 x 500 µL vials
Reagent Solution	FQA-DS-RGT	1 x 500 µL vial	1 x 1000 µL vial	2 x 1000 µL vial
Dilution Buffer	FQA-DIL	1 x 10 mL bottle	1 x 20 mL bottle	2 x 20 mL bottle
Microplate	FQA-PLT	1 plate		2 plates

Required Materials (NOT INCLUDED)

1. Nuclease-free Tris-EDTA buffer (or dH_2O)
2. Vortex Mixer
3. Tabletop centrifuge
4. Pipettes and nuclease-free pipette tips (barrier tips recommended)
5. Serological pipette and nuclease-free glass tips
6. Nuclease-free 15 mL conical tubes and 1.5 mL microcentrifuge tubes
7. Plate reader capable of reading fluorescence Ex/Em = 490/525 nm

General Considerations

1. Read this manual carefully before the experiment.
2. Allow assay components to equilibrate to room temperature before use.
3. Use calibrated pipettes and nuclease-free centrifuge tubes, conical tubes, and pipette tips.

Prepare Assay Buffer

***Note**: Protect Reagent Solution and completed Assay Buffer from light. Allow all reagents to equilibrate to room temperature before use. Spin down before opening.*

1. Determine total volume of Assay Buffer to prepare as follows:
   $$\boldsymbol{Volume\ assay\ buffer} = (\#\ \boldsymbol{Samples} + \mathbf{2}\ \boldsymbol{Controls} + \mathbf{1}) \times \#\ \boldsymbol{Replicates} \times \mathbf{150}\ \mu\boldsymbol{L}$$
2. Prepare Assay Buffer by diluting Reagent Solution 20-fold in Dilution Buffer.
   ***Ex.** For 10 samples run in duplicate, user would prepare 3900 µL Assay Buffer (calculated above in step 1) by mixing 195 µL Reagent Solution and 3705 µL Dilution Buffer.*

Prepare Tests

***Note**: Avoid direct exposure to bright light sources during preparation.*

1. Label 1.5 mL centrifuge tubes with "50 ng Standard," "0 ng Standard," and sample name(s).
2. Add the following components to the correspondingly labeled tubes:
   ***Note**: Volumes shown below are for a single replicate. Volumes should be scaled up based on number of intended replicates and prepared in a single tube before dispensing into separate wells of the microplate.*

	50 ng Standard	0 ng Standard	Sample Tube(s)
Assay Buffer	150 µL	150 µL	150 µL
DNA Reference^{+}	50 µL	-	-
Negative Reference	-	50 µL	-
Sample	-	-	1 to 50 µL*
Tris-EDTA Buffer (or dH_2O)	-	-	0 to 50 µL*
Total Volume	200 µL	200 µL	200 µL

   ***** 1 to 50 µL sample volume can be used depending on expected sample concentration. If using less than 50 µL sample volume, add an appropriate volume of nuclease-free Tris-EDTA buffer or dH_2O to maintain total volume (per replicate) of 200 µL.*
3. Vortex on highest setting for 5 seconds. Spin down.
4. Dispense 190 µL to well(s) of microplate.

Read on Microplate Scanner

1. Set-up fluorescence measurement protocol with the following parameters:
   Excitation: 490 nm
   Emission: 525 nm
   Bandwidth: 17 nm (can be set between 10 nm and 20 nm)
   Optics/read position: Top
   Gain: Set gain so that signal intensity of highest dsDNA concentration is near the microplate reader's maximum.
2. Read plate (uncovered).
3. Export and save relative fluorescence unit (RFU) measurements to an excel file.

Data Analysis

1. Plot relative fluorescence unit (RFU) versus DNA mass of the two standards (0 ng and 50 ng DNA) using Excel or a statistical software package (e.g., Prism GraphPad).
   Note*: If Tween-20 or BSA was added to standards (See notes under Table 1), adjust concentration and resulting mass/well of standard 1 as necessary before proceeding with analysis.*
2. Model data with linear regression and determine the equation for the line of best fit.
3. Use the equation of the resulting line of best fit to extrapolate mass of unknown samples (in ng).
   Caution*: If extrapolated mass is above 50 ng, measurement should be redone with a lower sample volume to ensure accurate data.*
4. Determine concentration of unknown samples by dividing extrapolated mass by volume of sample.
   Ex. *The resulting sample concentration for a test in which 25* µ*L sample was added (per replicate) and the average extrapolated mass was 15 ng would be 0.6 ng/*µ*L.*

Assay Performance

Assay performance was determined over four replicates with Reagent Solution and Dilution Buffer of different lots.

Intra-assay CV%: ≤ 10%

Inter-assay CV%: ≤ 15%

Limit of Detection (LOD): 1.77 ± 0.74 pg/µL (Average ± S.E.M)

Limit of Quantification (LOQ): 5.35 ± 2.24 pg/µL (Average ± S.E.M)

Range: 0.25 to 50 ng (5 pg/µL to 50 ng/µL)

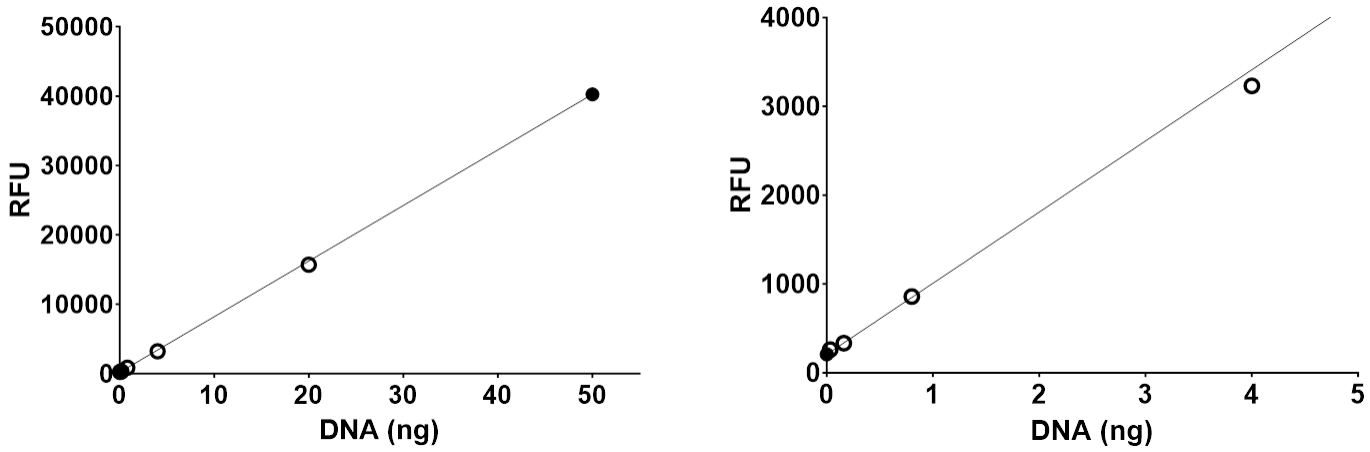

Figure 1. Representative data for 0 ng and 50 ng DNA standards (solid circles, ●) and a dilution series prepared from the 50 ng standard (hollow circles, ○). The right panel shows a magnified view of the bottom five dilutions for enhanced clarity.

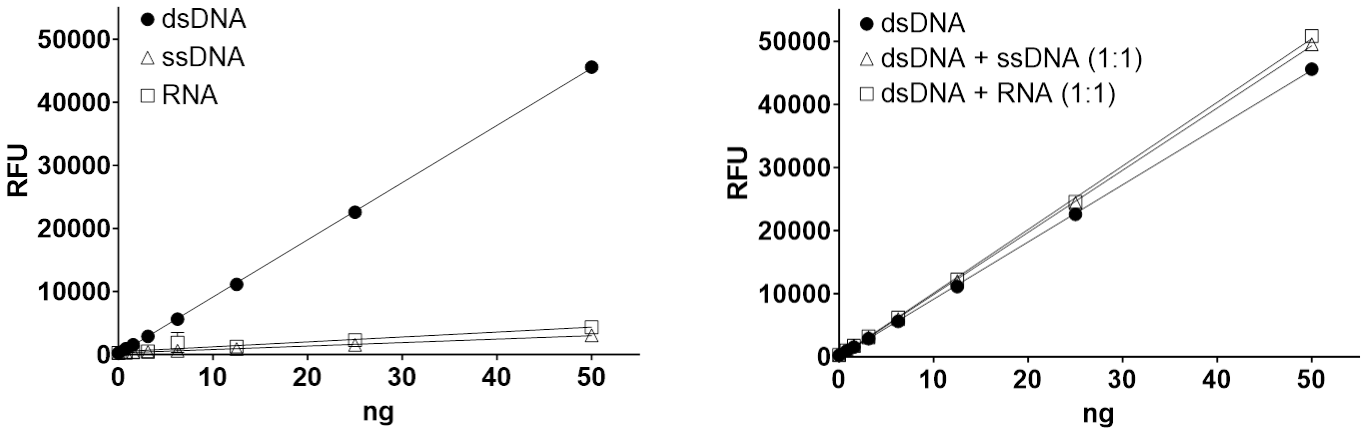

Figure 2. Sensitivity and selectivity of assay for double stranded DNA over single stranded DNA and RNA tested individually (left) and mixed in equal parts (right).

Table 1. Effect of contaminants on RayBio® dsDNA Quantification Assay across range of assay.

Contaminant	Final concentration in 200 µL assay	Concentration in 50 µL sample	Change In Signal[1]
NaCl	50 mM	200 mM	-6.4%
$MgCl_2$	5 mM	20 mM	-11.3%[2]
Sodium Acetate	30 mM	120 mM	-4.1%
Ethanol	1%	4%	2.5%
TRIzol™	0.1%	0.4%	-3.3%
Chloroform	0.1%	4%	2.3%
SDS	0.01%	0.04%	11.9%[3]
Triton™ X-100	0.025%	0.1%	-3.8%
Tween-20	0.1%	0.4%	[4]
BSA	10 mg/mL	40 mg/mL	[4]
Human IgG	0.1 mg/mL	0.4 mg/mL	-8.8%[2]
dNTP Mix	500 µM[5]	2 mM	3.8%

[1] Change measured relative to value obtained from assay performed without potential interfering contaminants. Results averaged across duplicate tests of 50 ng, 5 ng, and 0.5 ng dsDNA (50µL of 1000, 100, and 10 pg/µL dsDNA, respectively). Perturbation was less than 10% for most tests.

[2] Relative change above ±20% at highest DNA concentration tested. All other results are acceptable.

[3] Relative change above ±15% at lowest DNA concentration tested. All other results are acceptable.

[4] Substantial upward shift in standard curve values. Add component to each standard before proceeding with assay. Adjust concentration of standard 1 as necessary.

[5] Total concentration, with each dNTP present in equal parts (125 µM each).

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