Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

BioVision Incorporated

EZQuant™ dsDNA Quantitation Kit (Fluorometric)

10/14

(Catalog # K900-2000; 2000 assays; Store at -20°C)

I. Introduction:

Accurate and sensitive quantification of double stranded DNA (dsDNA) has wide applications. The major disadvantages of traditional absorbance based method for DNA quantitation include (1) lower sensitivity, (2) inability to distinguish between DNA and RNA, (3) interference due to contaminants commonly found in nucleic acid preparations and (4) relatively large contribution of nucleotides and single-stranded nucleic acid to the signal. BioVision's dsDNA Quantitation Kit is sensitive, non-radioactive and can specifically detect dsDNA in the presence of ssDNA, RNA, and free nucleotides. The assay is based on the fluorescent dsDNA-binding dye that specifically binds to dsDNA to generate fluorescence (Ex/Em = 480/530 nm), which enables the sensitive quantitation of small amounts of dsDNA (as low as 40 pg). This highly sensitive assay is ready as supplied for 96-well plate format and can be adapted to 384-well plate.

II. Application:

- Quantitation of dsDNA from viral DNA, miniprep DNA, genomic DNA, PCR amplification products etc.

III. Sample Type:

- dsDNA sample in solution

IV. Kit Contents:

Components	K900-2000	Cap Code	Part Number
TE Buffer (20X)	25 ml	NM	K900-2000-1
dsDNA Dye (200X)	1 ml	Blue	K900-2000-2
dsDNA Standard (100 µg/ml)	200 µl	Yellow	K900-2000-3

V. User Supplied Reagents and Equipment:

- 96-well white opaque plate with flat bottom
- Nuclease-free water
- Multi-well spectrophotometer (Fluorescence reader)

VI. Storage Conditions and Reagents Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **TE Buffer (20X):** Dilute to 1X with nuclease-free water (not provided). Warm to room temperature (RT) before use.
- **dsDNA Dye (200X):** For long-term storage, aliquot and store at -20°C. Avoid freeze/thaw. Before use, thaw at RT.

VII. dsDNA Assay Protocol:

1. **Samples Preparation:** Add 1-50 µl of dsDNA sample and adjust the volume to 50 µl/well with 1X TE buffer.
   Notes:
   a. Assay has the linear range from 50 pmol to 200 nmol dsDNA. If necessary, dilute the samples to make sure dsDNA amount fall within this linear range.
   b. For samples having significant background, prepare parallel sample well(s) as sample background control(s).
2. **Standard Curve:** Prepare 10 ng/µl of dsDNA Standard by adding 10 µl of dsDNA Standard (100 µg/ml) into 90 µl 1X TE buffer. Add 0, 2, 4, 6, 8 and 10 µl of 10 ng/µl dsDNA Standard into a series of wells in 96-well plate to generate 0, 20, 40, 60, 80, and 100 ng/well dsDNA Standard. Adjust the volume to 50 µl/well with 1X TE buffer.
   Note: For higher sensitivity, prepare 1 ng/µl dsDNA Standard by further diluting the 10 ng/µl dsDNA Standard. Add 10 µl of 10 ng/µl dsDNA Standard into 90 µl 1X TE Buffer and prepare Standards as described for 10 ng/µl dsDNA Standard to generate 0, 2, 4, 6, 8 and 10 ng/well dsDNA Standard.
3. **dsDNA Dye Incorporation:** Dilute dsDNA Dye (200X) to 2X using 1X TE Buffer. Prepare enough for dsDNA Standards, and sample wells. Add 50 µl of 2 X dsDNA Dye to each well. Protect the reaction from light. Gently shake plate for 5 min. on a shaker at RT.
4. **Measurement:** Measure fluorescence (Ex/Em = 480/530 nm).
5. **Calculations:** Subtract 0 Standard reading from all readings. Plot the dsDNA Standard Curve. If sample background control reading is significant, subtract sample background control reading from sample reading. Apply sample's corrected RFU to Standard Curve to get B ng of dsDNA in sample wells.

Sample dsDNA concentration (C) = B/V x Dilution Factor = ng/µl = µg/ml

Where: **B** is dsDNA amount in the sample well from Standard Curve (ng)
V is sample volume added into reaction well (µl)

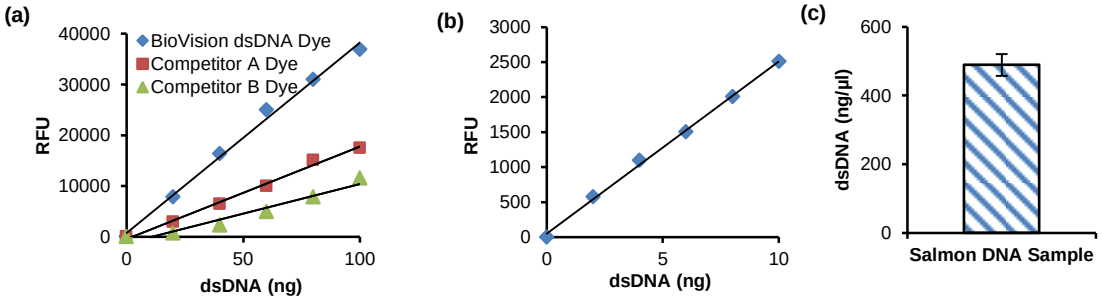

Figure: (a) dsDNA Standard Curve: comparison of BioVision's dsDNA Dye with Competitor A and Competitor B Dye. **(b)** dsDNA Standard Curve 0-10 ng range. **(c)** Measurement of Salmon DNA concentration using this kit.

VIII. RELATED PRODUCTS:

DNA Damage Quantification Colorimetric Kit (K253)
ApoBrdU Red DNA Fragmentation Assay Kit (K404)
ApoDIRECT DNA Fragmentation Assay Kit (K402)
Enhanced Apoptotic DNA Ladder Detection Kit (K130)
Genomic DNA Isolation Kit (K281)
Link-FAST™ 5 Minutes DNA Ligation Kit (K902)
ApoBrdU DNA Fragmentation Assay Kit (K401)
ApoBrdU-IHC DNA Fragmentation Assay Kit (K403)
Apoptotic DNA Ladder Isolation Kit (K170)
Quick Apoptotic DNA Ladder Detection Kit (K120)
Mitochondrial DNA Isolation Kit (K280)

FOR RESEARCH USE ONLY! Not to be used on humans.