DireCtQuant DNAQ USER GUIDE

Fluorescent DNA dye for easy quantification of DNA content in samples prepared with DireCtQuant 100ST /100W

For Research Use Only

Not Intended for any Animal or Human Diagnostic or Therapeutic Use

www.directquant.eu

CONTENTS

ABOUT Dire*Ct*Quant DNAQ

Dire*Ct*Quant DNAQ reagent is a fluorescent DNA dye for easy quantification of DNA content in samples prepared with **Dire*Ct*Quant 100ST** or **Dire*Ct*Quant 100W**.
Unlike mRNA or protein levels of different housekeeping (also known as normalization or reference) genes, DNA amount per cell (called also C-value which stands for constant-value, the haploid genome expressed in pg) is not changing in any experimental settings (with very limited exceptions) and is in linear relation with the number of cells in the sample. This makes it an ideal benchmark for reliable sample normalisation between samples of the same experiment as well as between samples across different experiments, *etc.*
The use of **Dire*Ct*Quant DNAQ** eliminates the necessity of the measurement of expression levels of reference genes in RT-qPCR or amount of housekeeping proteins in Western blot in order to normalise the data. Using this approach the reliability of the data is highly increased and the experimental work and cost significantly reduced.
As this method gives a very precise estimation of the total number of cells, it can also be used as a cell viability assay (e.g. if the experimental treatment changes the number of cells due to toxicity, division suppression, *etc.*).

TECHNOLOGY BEHIND Dire*Ct*Quant DNAQ

Dire*Ct*Quant DNAQ contains proprietary dye specially designed to selectively form complex with double stranded DNA in a sample prepared with **Dire*Ct*Quant 100ST** or **Dire*Ct*Quant 100W.** This allows the measurement of DNA concentration in the sample with fluorimetry based method; the excitation/emission maximum of the DNAQ-DNA complex is 495nm/530nm (see Figure 1). The presence of proteins, lipids and RNA in the samples prepared with **Dire*Ct*Quant 100ST/100W** does not interfere with the sensitivity of the DNA measurement. Equal amount of RNA or protein in solution are exhibiting just around 15% and 1% increase in the signal, respectively.

Dire*Ct*Quant DNAQ based method for DNA measurement is very fast (minutes) and can be easily adapted for variety of experimental setups as fluorescence plate reader, micro-fluorimeter or qPCR etc.

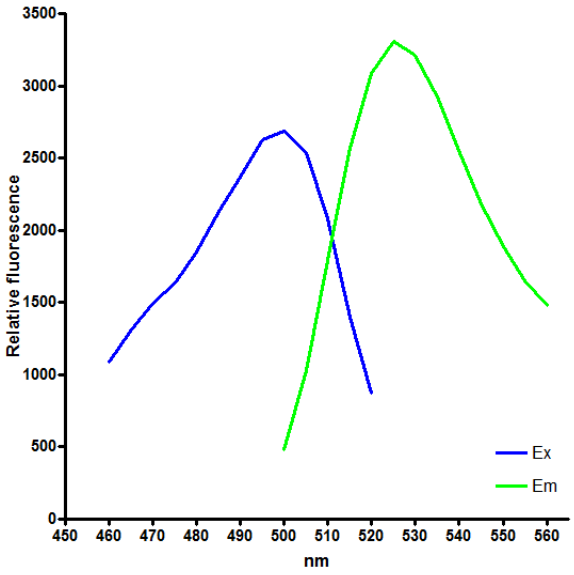

Figure 1. The excitation (blue) and emission (green) spectra of **Dire*Ct*Quant DNAQ-DNA** complex.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY USER

- Microliter/PCR plate
- Fluorescence plate reader, micro-fluorimeter or qPCR instrument
- DNA standard
- Nucelase-free water

REAGENT STORAGE

DireCtQuant DNAQ is shipped at ambient temperature, upon arrival store at -20°C. Once opened, store at 4°C for up to 1 month. Protect from unnecessary light exposure.

SAMPLE PREPARATION

In order to achieve proper measurement of the DNA content using **DireCtQuant DNAQ,** samples should be prepared using **DireCtQuant 100ST** or **DireCtQuant 100W**, for more details about sample preparation please refer to **DireCtQuant 100ST user manual** or **DireCtQuant 100W user manual**

DNA CONCENTRATION MEASUREMENT

1. **Prepare the working solution of DireCtQuant DNAQ by diluting it 100 times with nuclease free water (e.g. 10 µl of the stock solution with 990 µl of water).**
   * **NOTE:** ***DireCtQuant DNAQ*** *is supplied in DMSO. Bring the stock solution to room temperature in advance and homogenize well before use.*

   ! **IMPORTANT:** *Always prepare the working solution of* ***DireCtQuant DNAQ*** *just before use, as the working solution is stable for only a few hours. Keep it protected from light.*

2. **Prepare standard curve using DNA standard with known concentration (not provided).**
   ! **IMPORTANT:** For DNA standard purified DNA with known concentration, dissolved in pure nuclease free water without additives (TRIS, EDTA, etc.) should be used.

   ! **CAUTION:** Do not use supercoiled plasmids as a standard DNA! You can use lambda DNA or linearized and purified plasmid, purified genomic DNA etc..

   ! **IMPORTANT:** *For diluting DNA standard use* ***DireCtQuant 100ST*** *or* ***DireCtQuant 100W*** *depending of the buffer used to prepare the samples.*

   * **NOTE:** *The recommended range of the standard curve is 1 to 25 ng/µl DNA (equivalent to approx 200 to 5000 mammalian cells/µl).*

3. **Aliquot 20µl of the working solution (prepared in step 1.) in tubes/microplate wells enough for all standards and samples. Add and mix well 20 µl of the sample or standard dilution**
   * **NOTE:** *If a microplate reader is used mix 100 µl sample plus 100 µl working DireCtQuant DNAQ solution per a well of a 96-well plate. For any other plate formats, scale appropriately.*

* **NOTE:** *Protect the samples from long term exposure (more than minutes) to light.*

4. **Measure the fluorescence of the samples and the standards (Use excitation λ_{max}=495nm and emission λ_{max}=535nm) on microfluorimeter, fluorescence plate reader. You can also use SYBR / FAM channel of a Real-Time PCR instrument.**

   * **NOTE: :** *If Real-Time PCR system is used set up the machine for single measurement at 20°C and look at the raw data for SYBR / FAM channel.*

5. **Create a standard curve using the data from standard DNA serial dilutions and extrapolate the concentration of your sample.**

   * **NOTE:** *Depending on the technology of the instrument used the resulting standard curve could be straight line or polynomal function (see figure 2). Use the proper curve fitting method and expect to achieve r^2>0.995.*

   * **NOTE:** *For extrapolating the sample concentration from polynomial function you can use DNAQ template .*

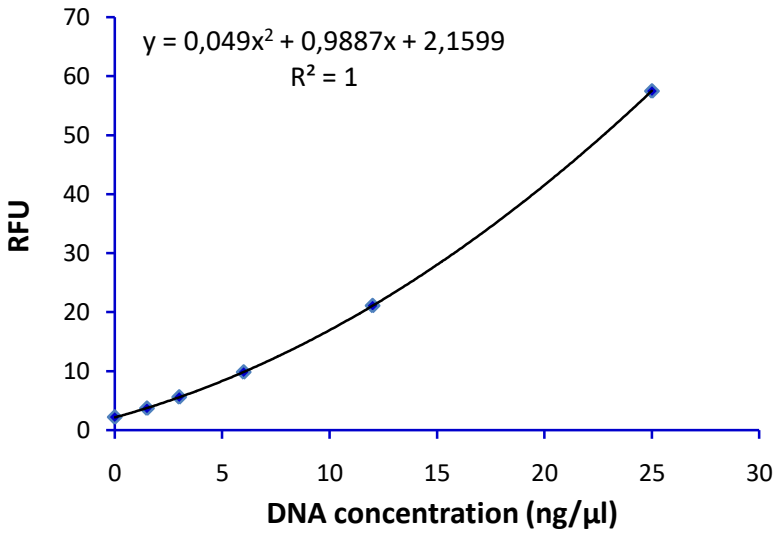

Figure 2. Example standard curve prepared with serial dilutions of 25 ng/µl DNA (0 ng/µl; 1.56 ng/µl; 3.13 ng/µl; 6.25 ng/µl; 12.5 ng/µl; 25 ng/µl) and fitted with polynomial function

6. **If a sample has a concentration higher than the most concentrated point of the standard curve dilute the sample (from step 3) further, using the corresponding reagent used for sample preparation (DireCtQuant 100ST/100W).**

CALCULATING NUMBER OF CELLS PER µL AND ADJUSTING ALL SAMPLES TO COMMON CONCENTRATION

Analysing equal amount of cells per RT-qPCR reaction for every sample is eliminating the requirement of measuring reference gene expression. Using reference gene expression in order to adjust for the loading differences of different samples has been always questionable as the expression of any gene can be influenced of the experimental conditions. The published guidelines (MIQE) require the usage of multiple reference genes simultaneously, which considerably increases the workload and cost of the experiments.
The same approach can be used for loading equal amount of cells per line in Western blot analysis, eliminating the necessity of detection of the housekeeping gene, which also can be regulated in particular experiment.
This workflow greatly simplifies the experimental work, provides substantial cost reduction and significantly improves the reliability of the analysis. For adjusting of the sample volumes always use the reagent used for the initial solubilisation of the sample (**Dire*Ct*Quant 100ST** or **Dire*Ct*Quant 100W**). Do not use water or another "buffer" which can interfere with the structure of the reagent and its properties.

1. **Calculate the number of cells per µl of Dire*Ct*Quant 100ST/100W extract. Assume that the cells in your sample are with diploid genome. Human genome contains 7 pg of DNA, mouse genome - 6.5 pg and rat genome - 6.9 pg of DNA.**

   * **NOTE:** *For other species and updated values of genome size please consult: www.genomesize.com – for animal reference values or www.kew.org/genomesize/homepage.html -for plant reference values.*

2. **Take into account if dilution is used during preparation of the sample.**

   ❖ **Example:** *Extrapolated concentration of a DNA sample is **6.9 ng/µl**.*
   *If rat cells/tissue has been used (**6.9 pg DNA/diploid cell**)*
   ***1pg** (picogram) **= 0.001ng** (nanogram)*
   *Number of cells per µl of the extract = 6900 pg/µl **/** 6.9 pg/cell = **1000 cells/µl**.*

3. **Dilute all your samples with appropriate amount of DireCtQuant 100ST/100W so that they all contain the same concentration of cells per µl (we recommend 1000 to 2000 cells/µl).**

❖ **Example:** *Sample **X** and **Y** are both prepared with **DireCtQuant 100ST** and are both with total volume of 200 µl.*
*Calculated number of cells per µl for sample **X** is 1000 cells/µl and for sample **Y** is 1250 cells/µl.*
*Using simple rule ([C]x / Vx = [C]y / Vy) is easy to calculate the desired final volume of sample **Y**:*

1000 / 200 = 1250 / Vy;
5 = 1250 / Vy;
Vy = 1250 / 5
Vy = 250 µl

*Adding 50 µl of **DireCtQuant 100ST** to sample **Y** will adjust the concentration to 1000 cells/µl.*

GENERAL STATEMENT

Handle our products in accordance with safe laboratory practices: *wear* suitable *protective gloves, eyewear* and *clothing*.

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In case of any doubt please feel free to contact DireCtQuant team at:
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