Solis BioDyne
Data Sheet

NGS qPCR Library Quantification Kit with EvaGreen®
For Illumina sequencing platforms

Cat. No.	Rxn (20 µl)	Rxn (10 µl)
QUANT-01-00500	500	1000

Kit composition	Vol.
5x HOT FIREPol® EvaGreen® qPCR Library Quantification Mix	2000 µl
50x Illumina Primer Premix	200 µl
Six 450 bp DNA Standards (10-fold serial dilutions)	100 µl (sufficient for 25 times)

Kit Description:
NGS qPCR Library Quantification Kit with EvaGreen® for Illumina sequencing platforms is a ready-to-use kit for quantifying amplifiable molecules in DNA library. Since qPCR only measures molecules that have sequencing adapter it prevents under or overestimation of library concentration, which may lead to poor results in sequencing reaction.
Kit comprises all the components necessary to perform library quantification with qPCR: 5x HOT FIREPol® EvaGreen® Library Quantification Mix, 50x Illumina Primer Premix and DNA Standards (six 450bp in 10-fold serial dilutions).

Applications:
- Suited for quantification of libraries
- Broad range of concentrations
- Suitable for wide range of GC concentrations

Suitable qPCR Platforms:
5x HOT FIREPol® EvaGreen® Library Quantification Mix is compatible with all qPCR platforms regardless of ROX requirements.

Component Specifications:
5x HOT FIREPol® EvaGreen® Library Quantification Mix is an optimised ready-to-use solution for real-time NGS library quantification, incorporating EvaGreen® dye. It comprises all the components necessary to perform qPCR: HOT FIREPol® DNA Polymerase, ultrapure dNTPs, $MgCl_2$ and EvaGreen® dye. Colour of qPCR mix is blue to provide more comfortable plate setup.

50x Illumina Primer Premix: Primers are complementary to Illumina library adaptors.

Six 450bp DNA Standards (10-fold serial dilutions) predispensed standards consist of a linear dsDNA target that includes Illumina library adapter sequence.

Standards provided:

450bp Standard	ds DNA concentration (pM)
450bp Std 1	20
450bp Std 2	2
450bp Std 3	0.2
450bp Std 4	0.02
450bp Std 5	0.002
450bp Std 6	0.0002

EvaGreen® Dye:
EvaGreen® is a DNA-binding dye with many features that make it a superior alternative to SYBR® Green I for qPCR. Apart from having similar spectra, EvaGreen® has three important features that set it apart from SYBR® Green I: EvaGreen® has much less PCR inhibition, is extremely stable dye and has been shown to be nonmutagenic and noncytotoxic. EvaGreen® is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM.

Shipping and Storage conditions:
Routine storage: -20ºC
Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of NGS qPCR Library Quantification Kit with EvaGreen®

Quality control:
Each lot of 5x HOT FIREPol® qPCR EvaGreen® Library Quantification Mix is functionally tested via qPCR.

Safety warnings and precautions:
This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

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Detailed Protocol:

1. **Step: Preparation of Reagent Mix**
   - Choose final volume of your qPCR reaction (20 µl or 10 µl).
   - Thaw reagents and mix thoroughly by vortexing 10 sec.
   - Prepare Reagent Mix by adding components into 5x HOT FIREPol® EvaGreen® qPCR Library Quantification Mix tube in order pointed out in Table 1 taking into account final reaction volume.
   - Mix well but do not vortex vigorously!

Table 1: Preparation of Reagent Mix depending on the reaction volume

Component	Final rxn vol 20µl	Final rxn vol 10 µl
5x HOT FIREPol® EvaGreen® qPCR Library Quantification Mix	2000 µl	2000 µl
PCR Grade Water	5800 µl	3800 µl
50x Illumina Primer Premix	200 µl	200 µl

2. **Step: Library sample preparation.**
   - Prepare 1:1000 (optionally also 1:2000, 1:4000, 1:8000) dilution of dsDNA library using 10mM Tris-HCl (pH 8.0) +0.05% Tween 20

3. **Step: Plate setup.**
   - Distribute 16 µl (final rxn vol 20 µl) or 6 µl (final rxn vol 10 µl) to each well of a qPCR plate. Add 4 µl of DNA Standard (450bp), library DNA sample or water (for NTC) to each well as shown in Table 2.
   - qPCR reactions for standards and library dilutions should be set up in triplicate.
   - Seal the reaction plate and centrifuge briefly to collect all components in the bottom of the wells.

Table 2: qPCR plate setup

Component	Final rxn vol 20 µl	Final rxn vol 10 µl
Reagent Mix	16 µl	6 µl
DNA Standard or Library DNA	4 µl	4 µl

4. Place the plate into qPCR instrument and run following program:

Cycle step	Temp.	Time	Cycles
Initial denaturation	**95ºC**	**12 min**	1
Denaturation	95ºC	20 s	40-45
Annealing	60ºC	30 s	
Elongation	72ºC	40 s	

- **IMPORTANT:** To activate the polymerase, include an incubation step **at 95ºC for 12 minutes** at the beginning of the qPCR cycle.

5. **Step: Analysis**
   - Confirm efficiency for standards and library (90 – 110%)
   - Determine concentration of the library based on the standard curve
   - Calculate library concentration taking into account length difference between standards and library fragments.

Average library concentration (pM) x 450bp / average fragment length (bp) = adjusted library concentration (pM)

   - Calculate the concentration of undiluted library by taking into account dilution factor (1:1000)

Adjusted library concentration x 1000 = library stock concentration

   - Prepare appropriate dilution of the library using calculated library stock concentration (10 – 13 pM)