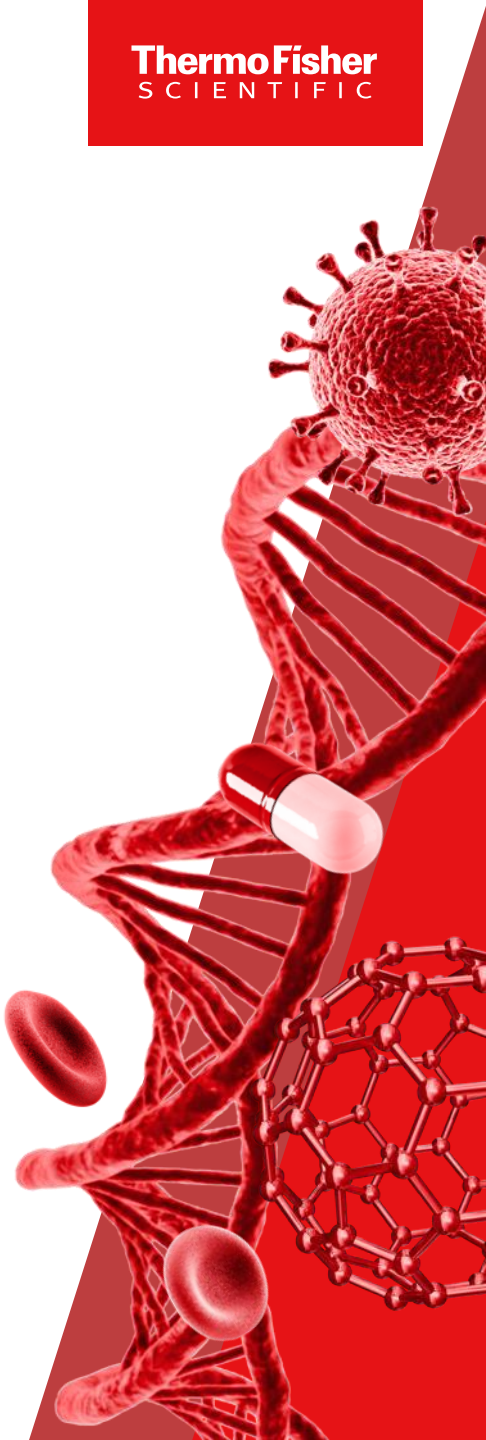
Qubit™ NGS Library Quantification Assay Kit

For selective quantification of sequencing-ready Illumina library molecules containing both P5 and P7 adapters, delivering direct molar concentration



Assay introduction

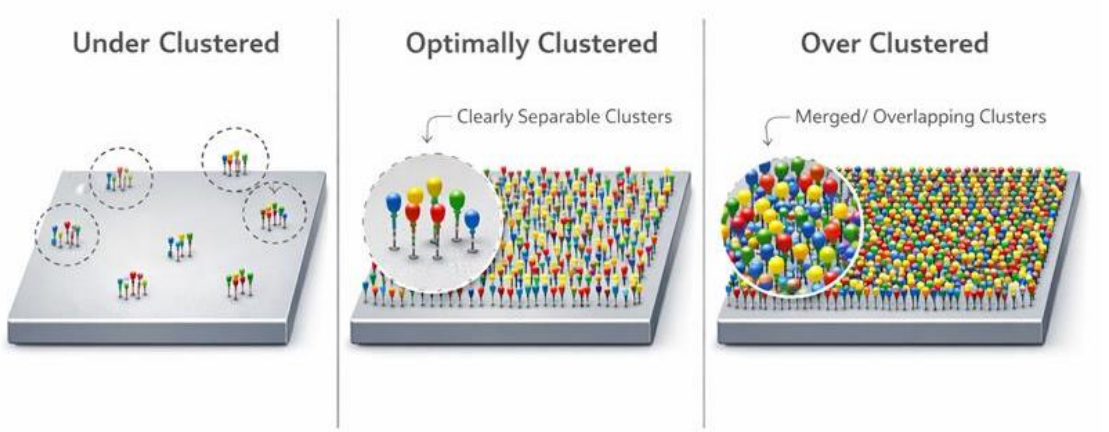
Invitrogen™ Qubit™ Flex NGS Library Quantification Assay Kit is designed to selectively measure sequencing-ready Illumina library molecules containing both P5 and P7 adapters enabling optimal cluster density during sequencing.

Key benefits of the Qubit NGS Library Quantification Assay Kit

- ✓ Selective molar concentration of dual-adapted library molecules
- ✓ Broad dynamic range (0.8 to 46 nM) with no dilution required
- ✓ Quantitation agnostic of library size
- ✓ Designed for use with Qubit™ Flex Fluorometer and fluorescence microplate readers
- ✓ Compatible with automation-friendly systems (e.g. KingFisher™ instruments)

Library quantification is critical to NGS

Nucleic Acid Purification	DNA / RNA Quant	Library Prep	Library Quant	Manual Normalization and Pooling	Sequencing



- **Under-clustering** reduces usable reads (total data output). Too low cluster density can sometimes cause image focus errors and sequencing artifacts.
- **Over-clustering** creates image analysis problems leading to lower Q30 scores, clusters passing filter, and data output. In some cases, over clustering can even lead to complete run failure.
- Library quantification is critical for successful multiplexing because it directly determines how evenly the samples will be represented in the final sequencing data.

Determining the number of sequencing-ready molecules following library preparation is a critical step in the Illumina NGS workflow and strongly influences the success of a sequencing run.

Common methods for library quantitation

	Qubit dsDNA HS Assay	qPCR Library Quantification Kit	Qubit NGS Library Quantification Assay
Method	Fluorometry Measures the enhanced fluorescence of a dye upon binding to DNA.	qPCR Uses capillary electrophoresis of DNA fragments for size estimation, intercalating dyes for quantification	Fluorometry Relies on selective capture and detection using sandwich hybridization approach
Concentration Output	Mass	Mass	Molarity
Library Sizing	Required	Required	Not Required
Sample Dilution	Optional	Required	Not Required
Workflow time	15 min	2.5 hours	45-60 min
Selectivity	No	Yes	Yes
Dynamic range	0.02 – 100 ng/ul	0.0002 – 20 pM (Dilution required)	0.8 – 46 nM (Dilution not required)

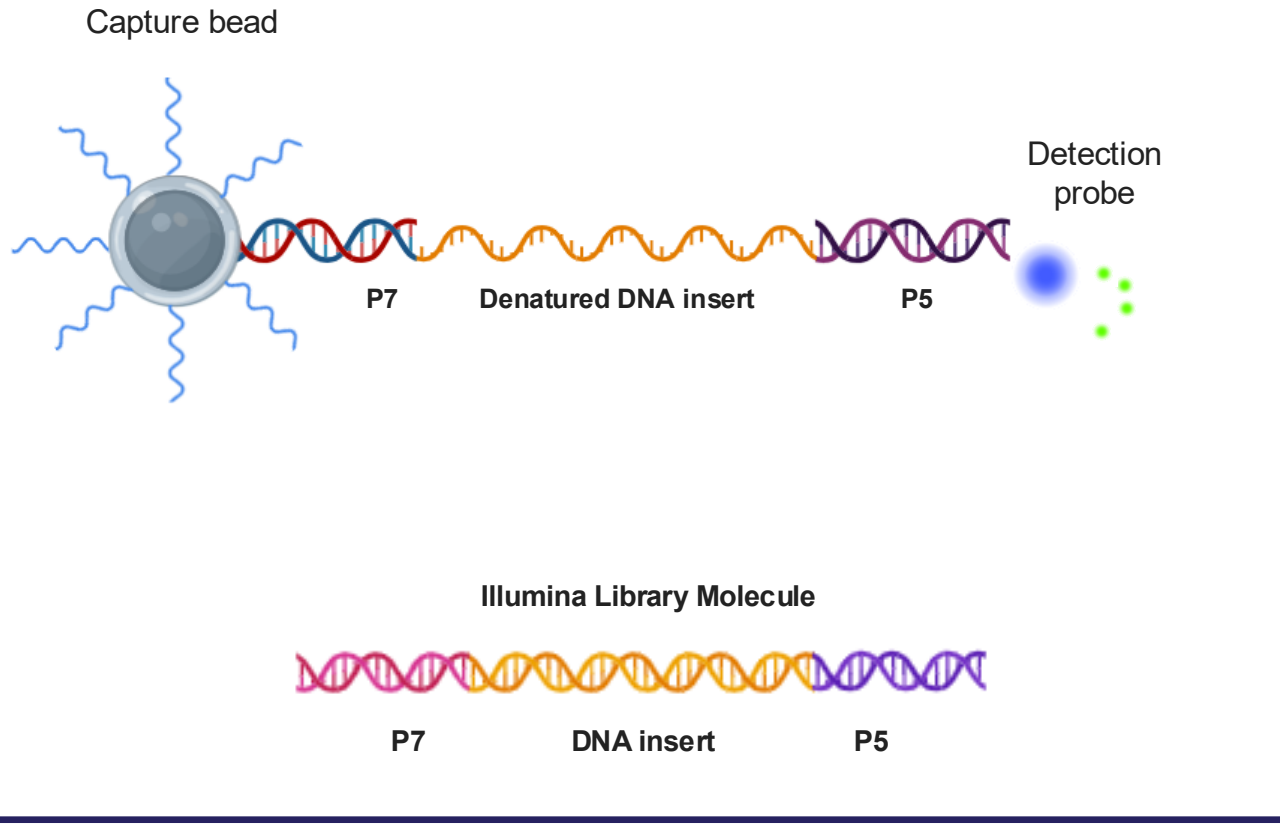
Qubit NGS Library Quantification Assay offers molar quantification mitigating the need for library sizing.

Side-by-side workflow comparison

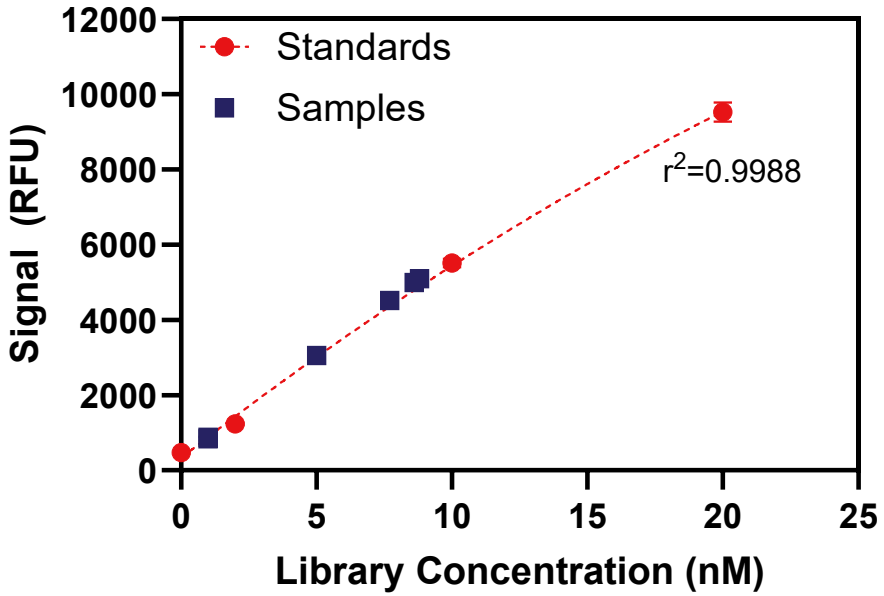
		Reagent preparation	Library dilution	Set up	Run assay	Data analysis	Total workflow
 qPCR	Hands-On	5 min.	10 min.	25 min.	1 min.	10 min.	**51 min.**
	Total	5 min.	10 min.	25 min.	60 min.	10 min.	**1 hr. 50 min.**
 Qubit NGS assay	Hands-On	5 min.	-	5 min.	12 min.	2 min.	**24 min.**
	Total	5 min.	-	10 min.	35 min.	2 min.	**52 min.**

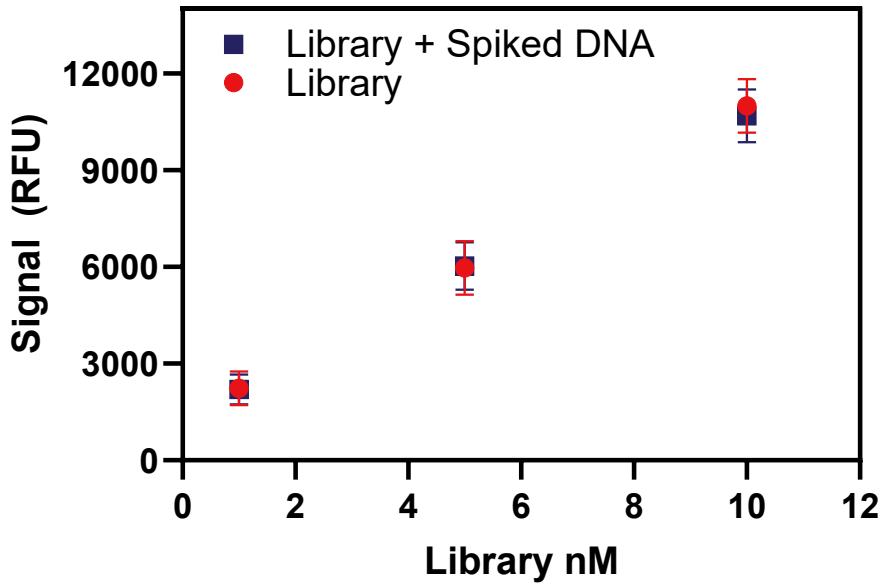
Assay background

- Illumina library molecules containing both P5 and P7 adapters are captured on magnetic beads conjugated with P7 oligos.
- P5-conjugated NGS Detection Probe hybridizes to the complementary P5 end of the captured libraries.
- Fluorescence substrate generates a fluorescent signal proportional to the amount of P5/P7-adapted library present.



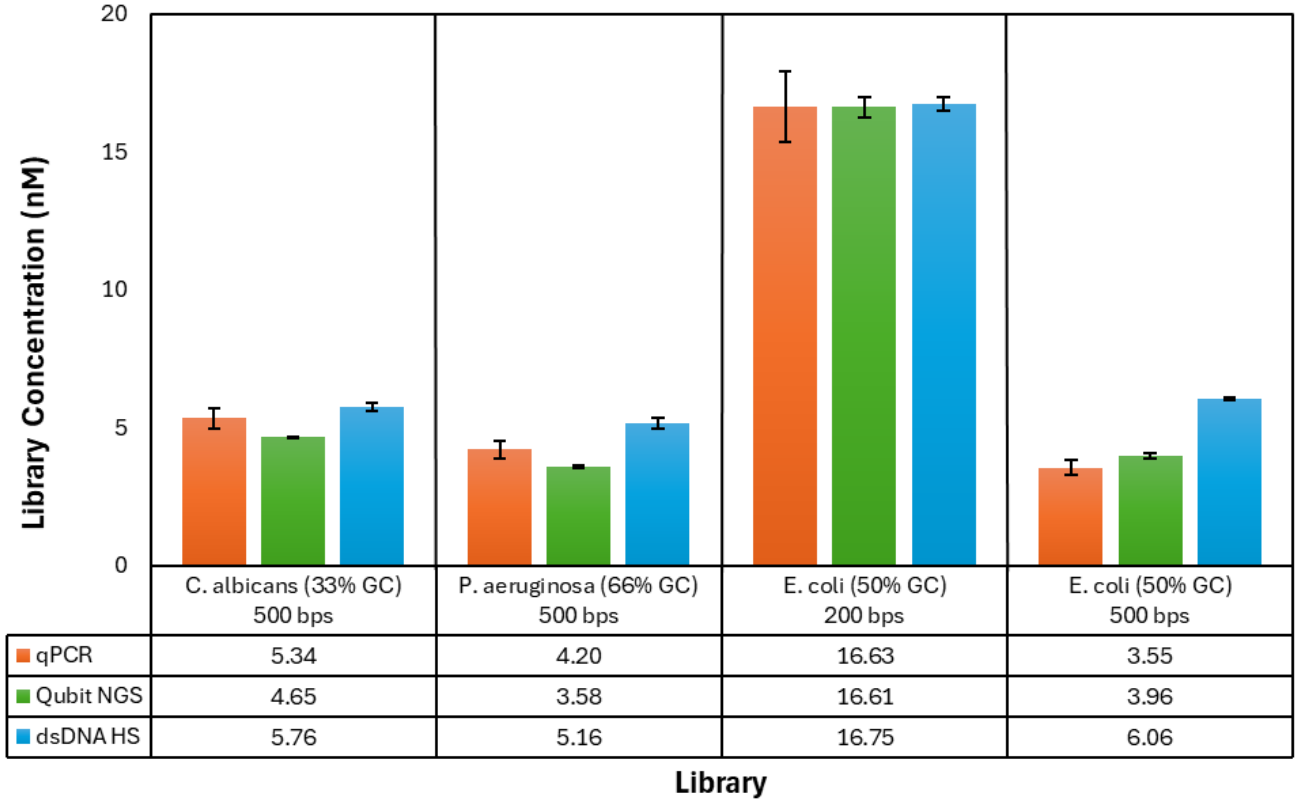
Selective for *only* P5/P7 adapted library DNA





- The standards are designed for quantifying sequencing-ready library samples and generating nM concentration output (left).
- The assay selectively detects only fully adapted library molecules containing both P5 and P7 and is not affected by up to equimolar amounts of unadapted DNA (right).

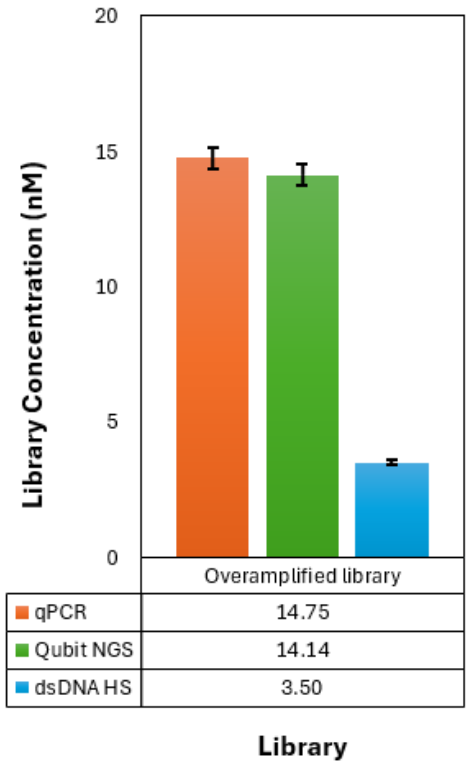
Accurate library quantification comparable to qPCR

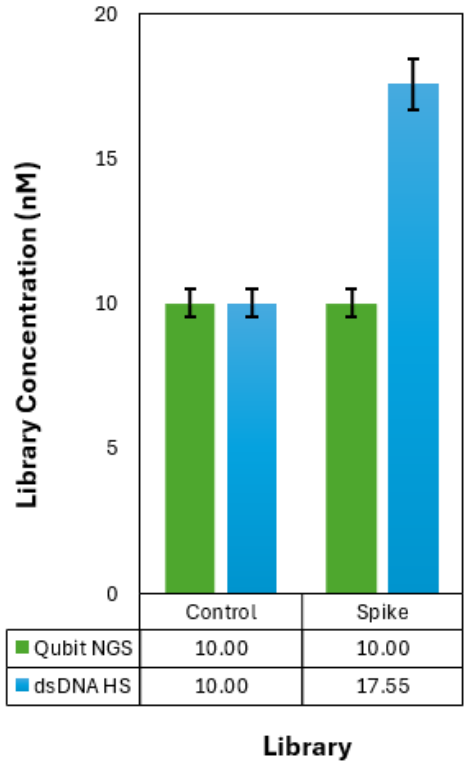


	C. albicans (33% GC) 500 bps	P. aeruginosa (66% GC) 500 bps	E. coli (50% GC) 200 bps	E. coli (50% GC) 500 bps
qPCR	5.34	4.20	16.63	3.55
Qubit NGS	4.65	3.58	16.61	3.96
dsDNA HS	5.76	5.16	16.75	6.06

- The Qubit NGS Library Quantification Assay reliably quantifies standard library samples with accuracy comparable to qPCR and Qubit, while requiring less than half the time of qPCR and fewer dilution steps.

Specific use-cases for the Qubit NGS Library Quantification Assay





- The Qubit NGS Assay can provide more reliable quantification than the Qubit dsDNA HS Assay for overamplified libraries (left) or libraries contaminated with improperly adapted DNA (right).

Assay kit components

Material	Invitrogen™ Qubit™ NGS Library Quantification Assay Kit (96 reactions, Cat. No. Q34250)
Capture Beads	1 mL
Hybridization Buffer	24 mL
Library Standards (0, 2, 10, 20nM)	4 vials (200 µL per vial)
1N Sodium Hydroxide	10 mL
Detection Probe	125 µL
Wash/Reaction Buffer	50 mL
Fluorescent Substrate	25 µL

Entire kit can be stored at 2–8°C. NaOH, standards, and substrate can be aliquoted and frozen at -20°C, Wash/Reaction buffer can be stored at RT.

- Kit is compatible with the Qubit Flex Fluorometer or a fluorescence-based microplate reader like the Varioskan LUX Multimode Microplate Reader
- Recommended input sample volumes are 2 – 5 uL

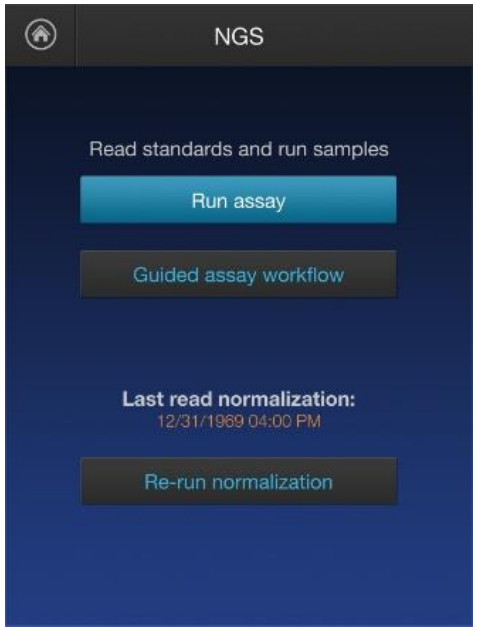
Assay workflow

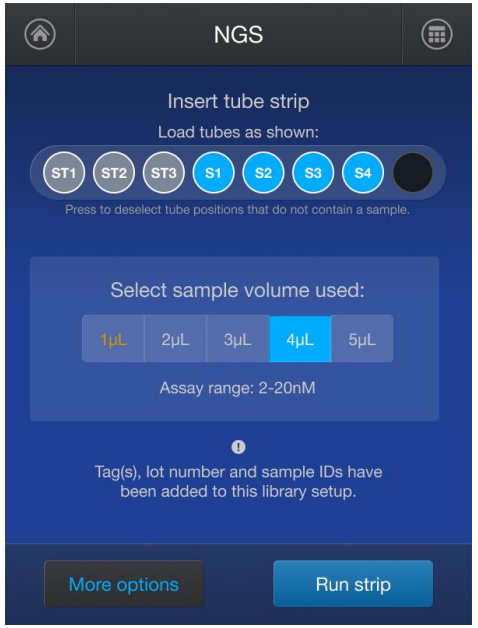
NGS Library Quantification Assay Workflow Steps	
1	Wash capture beads, prepare hybridization mix, dilute sodium hydroxide.
2	Add samples and standards to tubes and denature (5 min).
3	Hybridize at 40°C with shaking or occasional vortex (20 min).
4	Wash beads 3X with Wash/Reaction buffer and resuspend in 50 uL buffer.
5	Add 50uL diluted substrate and incubate at RT (5-10 min). Stop with 50uL sodium hydroxide.
6	Transfer to new strip tubes or well plate.
★	Read on a Qubit Flex Fluorometer or fluorescence microplate reader (Varioskan LUX or Varioaskan ALF)

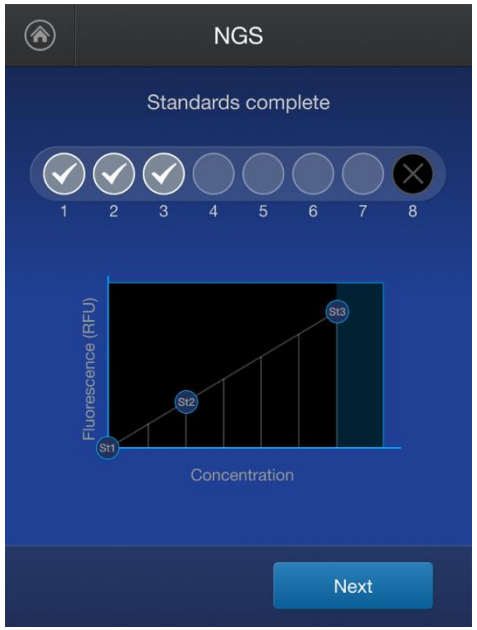
* Steps 3 – 6 are amenable for automation on a KingFisher™ Flex Purification System

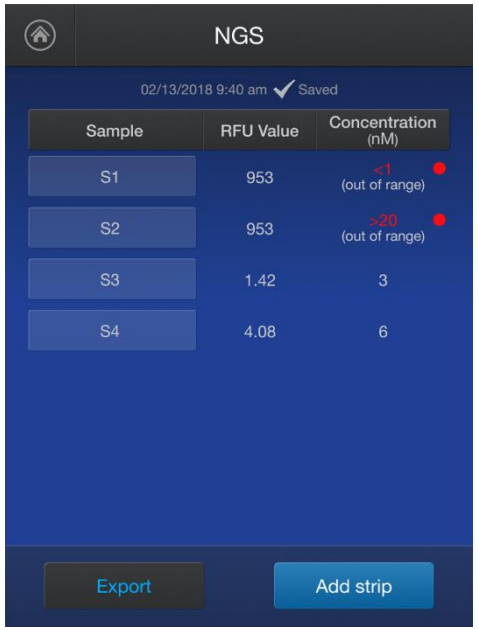
NGS Assay on Qubit Flex

New assay features include a **guided assay workflow**, **NGS assay calculator**, and **optimized data modeling** for concentration calculations and report outs.









1 **Select NGS assay**

2 **Run standards**

3 **Run Samples**

4 **Report Results**

Frequently asked questions

How can I order online?

- Qubit NGS Library Quantification kit (Cat. No. Q34250)
- Qubit Flex Pyrogen-Free Tube Strips (Cat. No. Q32893)

How should the product be stored?

- The entire kit can be stored at 2–8°C for 6 months.
- The Wash/Reaction buffer can be stored at room temperature.
- The 1N Sodium Hydroxide and Substrate should be aliquoted and stored at –20°C.

Does this new assay require a software update?

- Yes, the Qubit Flex software is available at thermofisher.com/qubitresources

Will this new assay be available on the Qubit 4 Fluorometer?

- No, because of the number of standards being used, the assay will only be available on the Qubit Flex Fluorometer.

If I am running the assay on a Qubit Flex, do I need the fourth standard?

- No, the fourth standard is only required for measurements on a fluorescence microplate reader to enable a quadratic curve fit.

Can I run different sample volumes on the same strip?

- No, the standards are always 4 uL and the samples volumes on each strip should be the same.

Do I have to normalize? How often?

- Normalization is required prior to the first use. This process will use the Qubit Flex System Verification Assay Kit (Cat. No. Q33254). Normalization references are stable for 12 weeks.

What type of NGS libraries can I quantify with this kit?

- Any Illumina library can be quantified with this kit, regardless of library preparation method.

Do I have to run the standards on each strip?

- The Qubit Flex requires the standards be run on each strip in positions 1-3.

Learn more at **thermofisher.com/qubitassays**

Thank you