SRM 2372:Human DNA Quantitation Standard.

How it Can be Used to Calibrate qPCR Measurements in Your Laboratory

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Disclaimers

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SRM 2372
Human DNA Quantitation Standard

Components

A: Male/single donor/RNased/NIST
B: Female/multiple donors/NIST
C: Mixture/male & female/commercial

Quantities supplied:
110 µL of Human Genomic DNA ≈ 50ng/µL

Certification

Decadic Attenuance (**Absorbance**) by a US National Reference Spectrophotometer
Homogeneity by a Cary 100 Bio Spectrophotometer
Validation of conventional [DNA] by Interlaboratory Study and NIST qPCR studies.

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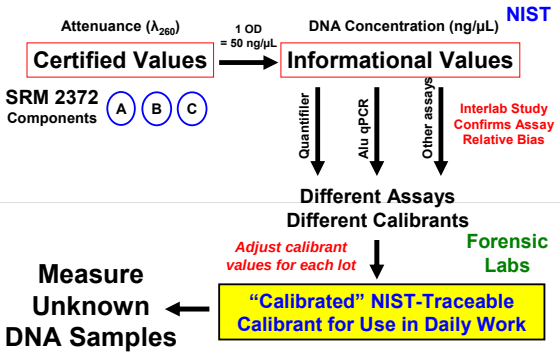
Overview of SRM 2372 Values and Use



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Nominal DNA Concentrations

Using 1 OD = 50 ng/µL double stranded DNA.

Informational Values

Component	Nominal [DNA], ng/µL
A	52.5
B	53.6
C	54.3

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So how do you use this SRM?

- You are going to calibrate your materials and make them NIST Traceable by using SRM 2372 to create the standard curve.
- How?
  - By analyzing **your materials** with **your DNA Quantification Methods** and assigning a [DNA] based on the values obtained **using SRM 2372 materials to generate your standard curve.**

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Examples of Value Assignment

- You have a tube of DNA you use for a Calibration Standard in your qPCR assay.
- Make Serial dilutions of this material to run in your qPCR value assignment assay:

  Serial dilutions: S1_1:10→ S2_1:5 →S3_1:2 →S4_1:2 (serial dilutions can be varied)
- **The SRM 2372 components are used as the calibration standards (Serial 1:2 dilutions)**.
- All samples and standards are analyzed in duplicate.

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qPCR plate setup

	1	2	3	4	5	6	7	8
A	A_52.5	A_52.5	B_53.6	B_53.6	C_54.3	C_54.3	S1	S1
B	A_26.3	A_26.3	B_26.8	B_26.8	C_27.2	C_27.2	S2	S2
C	A_13.1	A_13.1	B_13.4	B_13.4	C_13.6	C_13.6	S3	S3
D	A_6.6	A_6.6	B_6.7	B_6.7	C_6.8	C_6.8	S4	S4
E	A_3.3	A_3.3	B_3.4	B_3.4	C_3.4	C_3.4	NTC	NTC
F	A_1.6	A_1.6	B_1.7	B_1.7	C_1.7	C_1.7		
G	A_0.8	A_0.8	B_0.8	B_0.8	C_0.8	C_0.8		
H	A_0.4	A_0.4	B_0.4	B_0.4	C_0.4	C_0.4		

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Quantifiler Human results: value assignment

Dilution code	Serial Dilutions	qPCR Results	SD	Multiple by	[DNA]	SD
S1	1:10	**12.6**	0.58	**10**	**126**	5.8
S2	1:5	**2.9**	0.02	**50**	**145**	0.8
S3	1:2	**1.4**	0.01	**100**	**138**	0.5
S4	1:2	**0.7**	0.02	**200**	**137**	3.9

New assigned value is:
Average of [DNA] column

= 136 ng/µL

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Use of SRM 2372

- DO NOT freeze
  - Store at 4 °C. We know it is stable at this temperature
  - We will continue tracking stability
- DO NOT aliquot into new containers
  - We know about our containers; we do not know about yours
- Use the SRM materials to calibrate commercial and in-house materials.
  - **Not intended for daily use!**
  - Intended for **you** to evaluate each new calibrant lot

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Thank you for your Attention!!

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