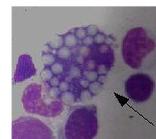


2. LIPSGENE® – GENE EXPRESSION QUANTIFICATION



LIPSGENE® GAPDH Kit

Intended use

The LIPSGENE® GAPDH Kit is intended for real-time PCR *in vitro* quantification of preformed glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) transcript cDNA, in total RNA or mRNA samples prepared from crude or purified cells of human, mice, rat, hamster, guinea pig and bovine origin. The RNA encoding *GAPDH* is a ubiquitously expressed moderately abundant messenger. It is frequently used as an endogenous control for quantitative RT-PCR analysis because its expression remains constant at different times and after experimental manipulation. The *GAPDH* gene has been reported to be constantly expressed in several heterogeneous tumour samples, tissues, cell lines and in CML. However, depending on the application type, the use of *GAPDH* as an endogenous control gene has to be checked prior to the application.

Kit contents

- ✓ Lyophilized oligonucleotide mix containing *GAPDH* cDNA specific primers and probes, PCR vessels containing stabilized synthetic *GAPDH* standard DNA (ready-to-use reference curves), sample PCR tubes (shipped at room temperature or together with taq polymerase on dry ice).
- ✓ Taq Polymerase, 10x PCR buffer, 50 mM Mg-chloride solution, PCR grade water, 10x ROX (shipped on dry ice).
- ✓ Sufficient to run 120 tests.

Note: Reverse transcription reagents available with a separate kit (M-MLV Reverse Transcription Kit, Cat.no. 1050001XX-050).

Performance assessment

The LIPSGENE® GAPDH cDNA Quantification Kit was evaluated together with total RNA isolated with "Reagent 14" using the Autogen 540 nucleic acids extraction robot (Integrated Separation Systems).

Assessment criterion	Sample type	Performance
Analytical sensitivity	Synthetic <i>GAPDH</i> DNA	≥5 copies/run
Linear range	Synthetic <i>GAPDH</i> DNA	7 logs

2. *LIPSGENE*[®] – GENE EXPRESSION QUANTIFICATION

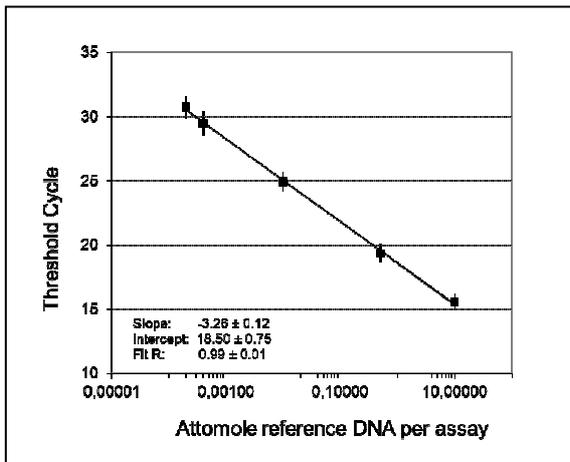
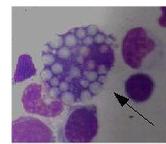


Figure 2.19: Repeatability of *GAPDH* cDNA quantification assay. The study was performed with synthetic *GAPDH* DNA specimen and 111 replicates for each concentration (ready-to-use *GAPDH* DNA standards; 10.010, 0.501, 0.010, 0.0004, 0.0002 attomoles per tube) on ABI PRISM SDS. The mean run data are included in the figure.

Typical run results

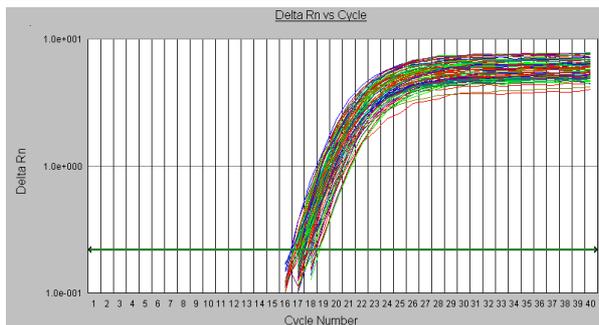


Figure 2.20: Quantification of *GAPDH* cDNA using an ABI PRISM SDS. Saturation curves. Quantitative analysis of *GAPDH* cDNA obtained from 43 different soft tissue sarcoma samples. Measurement performed in duplicate experiments.

Ordering information

Kit version	RP	ST	LP	LC	SC	SP	RS
IvD state	RUO	RUO	RUO	-	-	-	-
120 tests	1030102RP-120	1030102ST-120	1030102LP-120	-	-	-	-

RP = 0.2 ml regular profile 8-well/strip PCR tubes and cap strips (e.g. ABI PRISM[®] 7000/7300/7700 SDS [Applied Biosystems/Life Technologies], iCycler IQ[™]; IQ5 [Bio-Rad], MX3000P, Mx3005P [Stratagene]; Mastercycler[®] ep realplex [Eppendorf]); **ST** = Single 0.2 ml PCR Tube with attached cap (e.g. Rotor-Gene[™] 3000/6000, Rotor-Gene Q [Qiagen]; LineGene K [Bioer]); **LP** = 0.1 ml low profile 8-well/strip PCR tubes and cap strips (e.g. , 7500 Fast [Applied Biosystems/Life Technologies], MiniOpticon[™], CFX-96 [Bio-Rad]); **LC** = 20 µl LightCycler glass capillaries (LightCycler 2.x, Roche); **SC** = 25 µl SmartCycler tubes (e.g. SmartCycler[®] [Cepheid]); **SP** = 20 µl DX-12 reaction tubes (Spartan Dx-12); **RS** = 0.1 ml 4-well tube/strips (Rotor-Gene[™] 3000/6000, Rotor-Gene Q [Qiagen]).