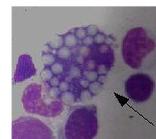


2. LIPSGENE® – GENE EXPRESSION QUANTIFICATION



2.2. REFERENCE GENE TRANSCRIPTS/ “HOUSEKEEPING GENES”

LIPSGENE® c-ABL Kit

Intended use

The LIPSGENE® c-ABL Kit is intended for real-time PCR *in vitro* quantification of preformed *c-ABL* transcript cDNA, in total RNA or mRNA samples prepared from crude or purified human white blood cells from blood or bone marrow aspirates. *c-ABL* gene expression was shown to have the highest correlation with the amount of a couple of fusion transcript cDNAs comprising those ones with the Ph⁺ translocation t(9;22). When measured in the same cDNA sample detection of *c-ABL* transcripts means confirming the RNA integrity and allows as well to correct for RNA load, cDNA synthesis efficiency, PCR inhibitors and possible analyte loss during long-time storage. According to the recommendations of the Program “Europe Against Cancer” (EAC), *c-ABL* is the reference gene for diagnosing MRD of leukaemia.

Kit contents

- ✓ Lyophilized oligonucleotide mix containing *c-ABL* cDNA specific primers and probes, PCR vessels containing stabilized synthetic *c-ABL* 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® *c-ABL* 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>c-ABL</i> DNA	≥5 copies/run
Linear range	Synthetic <i>c-ABL</i> DNA	5 logs
Robustness: failure rate of the system	K562 cDNA	0%

2. LIPSGENE® – GENE EXPRESSION QUANTIFICATION

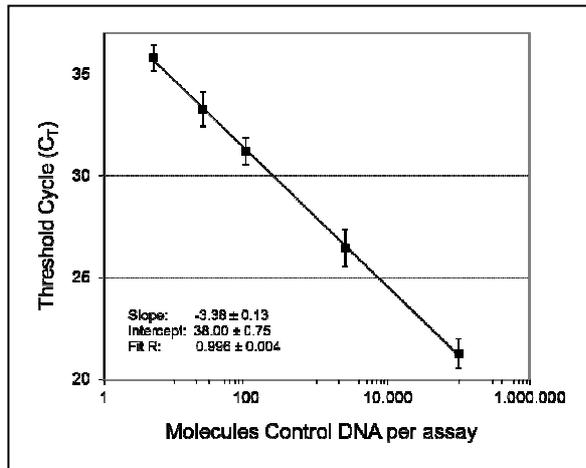
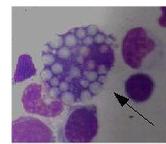


Figure 2.17: Repeatability of *c-ABL* cDNA quantification assay. The study was performed with synthetic *c-ABL* DNA specimen and 60 replicates for each concentration (ready-to-use *c-ABL* DNA standards; 10⁵, 2.5 × 10³, 10², 25, 5 copies per tube) on ABI PRISM SDS. The mean run data are included in the figure.

Typical run results

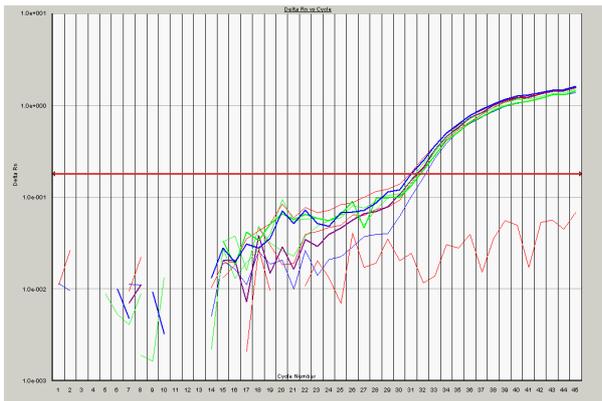


Figure 2.18: Quantification of *c-ABL* cDNA using an ABI PRISM SDS. Quantitative analysis of *c-ABL* transcripts in cDNA obtained from the K562 cell line. Analysis performed in 4 samples measured in duplicate. 2 µl of 1:10 diluted cDNA were analyzed using the LIPSGENE® *c-ABL* Kit.

Ordering information

Kit version	RP	ST	LP	LC	SC	SP	RS
IvD state	RUO	RUO	RUO	-	-	-	-
120 tests	1030101RP-120	1030101ST-120	1030101LP-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]).