

Ready-to-use nucleic acid Internal Controls DNA

INSTRUCTION MANUAL

For research use only, not for diagnostic use!

REF

Internal Control DNA Tubes and Real-Time Reagent Mix

for use with ABI PRISM[®] 7700/7000 SDS; iCycler IQ[™],
Rotor-Gene[™] 3000/6000 and SmartCycler[®]

50 tubes and reactions Cat.No. 0206200101
250 tubes and reactions Cat.No. 0206200102

for use with LightCycler[™]

50 tubes and reactions Cat.No. 0206200131
250 tubes and reactions Cat.No. 0206200132

Internal Control DNA Real-Time Reagent Mix

for use with ABI PRISM[®] 7700/7000 SDS; iCycler IQ[™],
Rotor-Gene[™] 3000/6000 and SmartCycler[®]

100 reactions Cat.No. 0204001101
500 reactions Cat.No. 0204001102

for use with LightCycler[™]

100 reactions Cat.No. 0204001131
500 reactions Cat.No. 0204001131

Internal Control DNA Tubes

100 tubes Cat.No. 0206200301
500 tubes Cat.No. 0206200302

-15°C
-40°C

Recommended storage temperature

aj ROBOSCREEN GmbH

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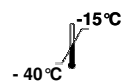
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INSTRUCTION MANUAL

Symbols and Abbreviations

	for X detections
	use by
	consult instructions
	catalogue number
	batch code
	manufacturer
	upper and lower limit of storage temperature

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CONTENTS

	Page
1. Introduction	3
2. Product description	3
3. General remarks	3
4. Storage and stability	4
5. Additional reagents and equipment required	4
6. Principle of the usage of <i>Internal Controls</i>	5
7. Contents of the ready-to-use nucleic acid <i>Internal Controls</i>	6
8. Experimental Procedure	8
8.1. Extraction of nucleic acids	8
8.2. Detection of <i>Internal Control</i> DNA using real-time PCR	8
8.2.1. Detection of <i>Internal Control</i> DNA using ABI PRISM 7700/7000 SDS or iCycler IQ instrument	8
8.2.2. Detection of <i>Internal Control</i> DNA using Rotor-Gene 3000/6000 instruments	10
8.2.3. Detection of <i>Internal Control</i> DNA using LightCycler instrument	11
8.2.4. Detection of <i>Internal Control</i> DNA using SmartCycler instrument	13
8.3. Interpretation of data	15
9. Sensitivity and precision, quality assessment	15
10. Carry-over prevention using UNG (optional)	16
Warranty Notice	16
References	16
Appendix 1: Recommended amplification conditions for different real-time instruments	17
Appendix 2: Calculation scheme for the preparation of an <i>Internal Control</i> reaction mix for use with ABI PRISM 7700/7000 SDS; iCycler IQ	18
Appendix 3: Calculation scheme for the preparation of an <i>Internal Control</i> reaction mix for use with Rotor-Gene 3000/6000	19
Appendix 4: Calculation scheme for the preparation of an <i>Internal Control</i> reaction mix for use with LightCycler	20
Appendix 5: Calculation scheme for the preparation of an <i>Internal Control</i> reaction mix for use with SmartCycler	21

1. Introduction

Corresponding to the *in vitro* diagnostic medical devices (IVD) AJ Roboscreen considers the growing demand for controls suited for standardized nucleic acids extraction, detection and quantification and offers a panel of ready-to-use DNA and RNA controls in order to improve customer quality assurance attempts and the reliability of laboratory data. The AJ Roboscreen stabilized ready-to-use DNA Internal Control is developed to be co-purified with several DNA targets particularly from body fluids. The sequence is that of a unique artificial gene, i.e. of completely synthetic origin. Introduced already on the sample preparation level the internal standard allows to control the efficiencies of DNA extraction and PCR amplification, respectively. False-negatives due to failed extraction or excess of inhibitors in the sample may be excluded when getting positive amplification results. All controls are exactly calibrated by a standardized quantitative real-time PCR protocol based on the AJ Roboscreen nucleic acids quantification platform technology.

The provided assay exploits the principle of quantitative real-time fluorescence PCR/RT-PCR. The increase in fluorescence is directly proportional to the specific target amplification during PCR.

2. Product description

Internal Control DNA Tubes and Real-Time Reagent Mix

For use with DNA extraction reagents and protocol of your choice

Eppendorf tubes (2.0 ml) coated with *in vitro* synthesized, calibrated DNA, real-time detection reagent mix in separate tubes, real-time PCR protocol

Internal control DNA Real-Time Reagent Mix

For use with AJ Roboscreen DNA extraction kits containing stabilized DNA Internal Controls (e.g. INSTANT Virus DNA Kit, Cat.No. 0209200401/02/03)

Real-Time Reagent Mix containing primers and probe for DNA Internal Control detection, lyophilized, real-time PCR protocol

Internal Control DNA Tubes

For use with DNA extraction reagents and protocol of your choice and detection of Internal Control DNA using separately available Reagent Mixes (e.g. Hepatitis B Virus/Internal Control Real-Time Reagent Mix, Cat.No. 0204100601/02)

Eppendorf tubes (2.0 ml) coated with *in vitro* synthesized, calibrated DNA

3. General remarks

This product is developed for research use only and the amplification protocol is optimized for ABI PRISM 7700/7000 SDS, Rotor-Gene 3000/6000 or LightCycler. The use of other real-time instruments like ABI PRISM 7900 may require additional optimization.

4. Storage and stability

After delivery at room temperature store AJ Roboscreen *Internal Control* reagents at –20°C. Protect Real-Time Reagent Mix, lyophilized, always from light and store at –20°C in the dark! Under these conditions reagents are usually stable for at least 6 month.

Prepared 4x or 5x reagent mixes, respectively, should be stored at 4-8°C in the dark. Under these conditions mixes are stable for at least 2 weeks.

All reagents not contained in the test kit should be stored according to the respective manufacturer's recommendations.

5. Additional reagents and equipment required

- **DNA polymerase[§] and corresponding 10x buffer concentrate**

[§] Recommended enzyme is the thermal activated AmpliTaqGold DNA polymerase (Applied Biosystems, Cat.-No. 4311806). The use with an other enzyme may require additional optimization experiments.

- **Real-time PCR instrument**

- Suited pipetting tools: recommended is an automated pipetting workstation, e.g. BIOMEK2000 (Beckman Instruments) particularly for high throughput analyzes (Ref. 1,2)
- 1.5 ml reaction tubes (e.g. Eppendorf AG, Hamburg)
- optical tubes, caps or covers for real-time PCR according to the real-time instrument used
- Barrier tips or Mastertips (e.g. Eppendorf) for manual pipetting
- Compression pad (Applied Biosystems, Cat.No. 4312639) for the use with real-time instrument supporting the 96-well format

For Cat.No. 0206200101/02 and 0206200131/32, respectively:

- **DNA extraction reagents and protocol of your choice**

For Cat.No. 0204001101/01 and 0204001131/32, respectively:

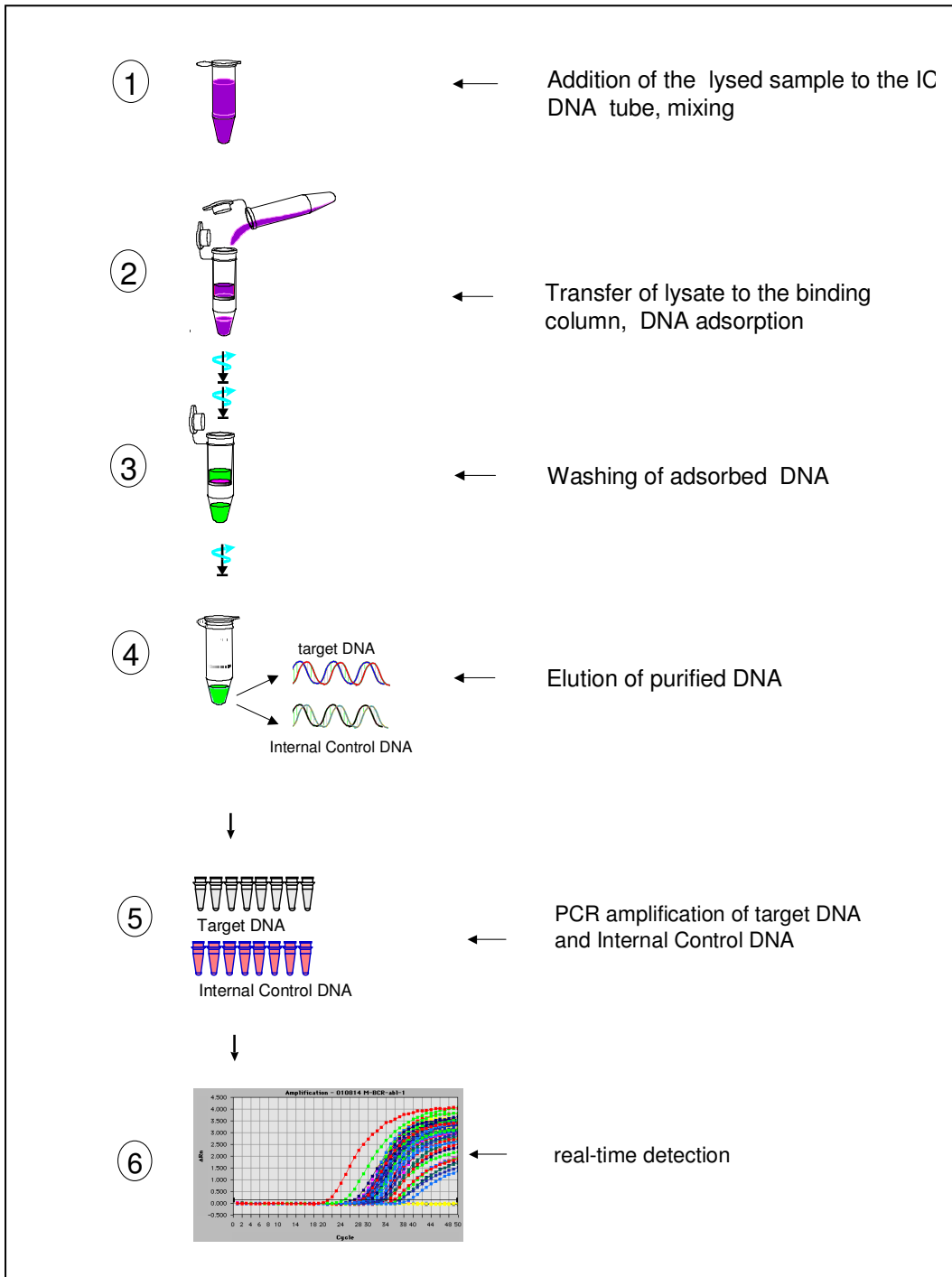
- **AJ Roboscreen DNA extraction kit containing stabilized DNA Internal Controls (e.g. INSTANT Virus DNA Kit)**

For Cat.No. 0206200301/02:

- **DNA extraction reagents and protocol of your choice**
- **Reagent Mixes for Detection of Internal Control DNA (e.g. Hepatitis B Virus/Internal Control Real-Time Reagent Mix)**

6. Principle of the usage of *Internal Controls*

Example: Extraction of DNA from samples using a spin filter membrane method



For use with AJ Roboscreen DNA extraction kits containing stabilized DNA Internal Controls (e.g. INSTANT Virus DNA Kit):
Extraction of *Internal Control* DNA is already performed with this kit.
Proceed from step 5 on.

5

7. Contents of the ready-to-use nucleic acid Internal Controls

Table 1: Contents of *Internal Control* DNA Tubes and Real-Time Reagent Mix (Cat.No. 0206200101/02 and 0206200131/32, respectively)

For use with ABI PRISM 7700/7000 SDS; iCycler IQ, Rotor-Gene 3000/6000 and SmartCycler				
	50 tubes and reactions (Cat.No. 0206200101)		250 tubes and reactions (Cat.No. 0206200102)	
	Contents	Preparation of the 5x reagent mix	Contents	Preparation of the 5x reagent mix
<i>Internal Control</i> DNA tubes, coated with <i>in vitro</i> synthesized, calibrated DNA	50 tubes		250 tubes	
<i>Internal Control</i> Reagent Mix, lyophilized, containing <i>Internal Control</i> primers, probe (5' Yakima Yellow) and dNTPs	2 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly	10 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly
MgCl ₂ (50 mM)	1x 1.0 ml		5x 1.0 ml	
10x PCR buffer, LC ②	1x 0.5 ml		5x 0.5 ml	
10x BSA solution ②	1x 0.5 ml		5x 0.5 ml	
PCR grade water	2x 1.5 ml		10x 1.5 ml	
10x Passive reference dye solution ①	1x 0.5 ml		5x 0.5 ml	
Manual	1		1	
For use with LightCycler				
	50 tubes and reactions (Cat.No. 0206200131)		250 tubes and reactions (Cat.No. 0206200132)	
	Contents	Preparation of the 4x reagent mix	Contents	Preparation of the 4x reagent mix
<i>Internal Control</i> DNA tubes, coated with <i>in vitro</i> synthesized, calibrated DNA	50 tubes		250 tubes	
<i>Internal Control</i> Reagent Mix, lyophilized, containing <i>Internal Control</i> primers, probe (5' Yakima Yellow) and dNTPs	2 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly	10 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly
MgCl ₂ (50 mM)	1x 1.0 ml		5x 1.0 ml	
10x PCR buffer, LC	1x 0.5 ml		5x 0.5 ml	
10x BSA solution	1x 0.5 ml		5x 0.5 ml	
PCR grade water	2x 1.5 ml		10x 1.5 ml	
Manual	1		1	

① only in kit versions for Detection of *Internal Control* DNA using ABI PRISM 7700/7000 SDS, or iCycler IQ instrument

② only in kit versions for Detection of *Internal Control* DNA using SmartCycler instrument

Table 2: Contents of *Internal Control* DNA Real-Time Reagent Mix (Cat.No. 0204001101/02 and 0204001131/32, respectively)

For use with ABI PRISM 7700/7000 SDS; iCycler IQ, Rotor-Gene 3000/6000 and SmartCycler				
	100 reactions (Cat.No. 0204001101)		500 reactions (Cat.No. 0204001102)	
	Contents	Preparation of the 5x reagent mix	Contents	Preparation of the 5x reagent mix
<i>Internal Control</i> Reagent mix, lyophilized, containing <i>Internal Control</i> primers, probe (5' Yakima Yellow) and dNTPs	3 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly	15 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly
MgCl ₂ (50 mM)	1x 1.0 ml		5x 1.0 ml	
10x PCR buffer, LC ②	1x 0.5 ml		5x 0.5 ml	
10x BSA solution ②	1x 0.5 ml		5x 0.5 ml	
PCR grade water	2x 1.5 ml		10x 1.5 ml	
10x Passive reference dye solution ①	1x 0.5 ml		5x 0.5 ml	
Manual	1		1	
For use with LightCycler				
	100 reactions (Cat.No. 0204001131)		500 reactions (Cat.No. 0204001132)	
	Contents	Preparation of the 4x reagent mix	Contents	Preparation of the 4x reagent mix
<i>Internal Control</i> reagent mix, lyophilized, containing <i>Internal Control</i> primers, probe (5' Yakima Yellow) and dNTPs	3 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly	15 tubes	add 200 µl PCR grade water per tube and incubate at 37°C for 20 min, vortex and centrifuge briefly
MgCl ₂ (50 mM)	1x 1.0 ml		5x 1.0 ml	
10x PCR buffer, LC	1x 0.5 ml		5x 0.5 ml	
10x BSA solution	1x 0.5 ml		5x 0.5 ml	
PCR grade water	2x 1.5 ml		10x 1.5 ml	
Manual	1		1	

① only in kit versions for Detection of *Internal Control* DNA using ABI PRISM 7700/7000 SDS or iCycler IQ instrument

② only in kit versions for Detection of *Internal Control* DNA using SmartCycler instrument

Table 3: Contents of *Internal Control* DNA Tubes (Cat.No. 0206200301 and 0206200302)

	100 tubes (Cat.No. 0206200301)	500 tubes (Cat.No. 0206200302)
	Contents	Contents
<i>Internal Control</i> DNA Tubes, coated with <i>in vitro</i> synthesized, calibrated DNA	100 tubes	500 tubes

8. Experimental Procedure

8.1. Extraction of nucleic acids

For use with Cat.No. 0206200101/02 and 0206200131/32, respectively (*Internal Control DNA Tubes and Real-Time Reagent Mix*) or for use with Cat.No. 0206200301/02 (*Internal Control DNA Tubes*)

Extract DNA as usual with an extraction kit from your supplier of choice. All protocols, i.e. both chemical extraction and extraction using spin filter membranes may be applied.

Important! Do not add sample (e.g. body fluids) directly to the AJ Roboscreen *Internal Control Tubes*. Ribonucleases potentially contained in the sample may hydrolyze the *Internal Control DNA*.
First mix your sample with a lysis reagent contained in the DNA extraction kit, then transfer the sample to the nucleic acid *Internal Control Tubes*. Mix thoroughly by vortexing and proceed as described in the manufacturer's manual of the DNA purification kit.

For use with Cat.No. 0204001101/02 and 0204001131/32, respectively (*Internal Control DNA Real-Time Reagent Mix*)

Extract DNA using AJ Roboscreen DNA extraction kit containing stabilized DNA *Internal Controls* (e.g. INSTANT Virus DNA Kit, Cat.No. 0209200401/02/03) and follow the manual of the kit.

8.2. Detection of *Internal Control DNA* using real-time PCR

8.2.1. Detection of *Internal Control DNA* using ABI PRISM 7700/7000 SDS or iCycler IQ instrument

Preparation of reagent mixes and samples

1. For preparation of the "5x reagent mix" please refer to **Table 1** or **Table 2**, respectively.

2. Prepare a final reaction mix as shown in **Table 4**. A proposed calculation scheme for the preparation of the reaction mix is given in **Appendix 2**.

3. Add appropriate aliquots of the reaction mix to 0.2 ml optical PCR tubes or strips considering the volume of sample to be added.

Caution! Do not exceed the final volume of 25 μ l.

4. After manual or automated dispensing of the reaction mix, add equal aliquots of extracted DNA samples to the tubes. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with an equal volume PCR-grade water.

5. Tailor an optical cover according to the number of used wells and seal the tubes carefully. Centrifuge at 200 x g for 1 min (1000 rpm) in a standard benchtop centrifuge. To avoid leakage during amplification, after placing on the wells tightly press on the cover and use a compression pad (Applied Biosystems, Cat.No.: 4312639) following the instructions of the manufacturer! **Always wear gloves when handle the tubes and covers!**

6. Select the wells containing non-target controls (“Blank”) and samples (“Unknown”) from the sample type pop-up menu. Choose the dye “JOE/VIC” from the dye layer pop-up menu.

7. Setup the amplification conditions as shown in **Table 5**.

Table 4: Pipetting scheme for the preparation of *Internal Control* reaction mix; calculated to achieve **a final PCR reaction volume of 25 µl in each tube.**

Reagents	<i>Internal Control</i> reaction mix [µl]	
	1 reaction	final conc.
PCR-grade water	❶	-
10x PCR buffer ❷	2.5	1x
10x Passive reference dye solution ❸	2.5	1x
MgCl ₂ (50 mM)	2.5	5 mM
5x Reagent mix (containing dNTP, primers and probe)	5.0	1x
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl) ❷	0.3	1.5 U per reaction

- ❶ Volume depends on the intended volume of DNA sample and the usage of passive reference dye solution; supplement to achieve **a final volume of 25 µl.** Calculation scheme see **Appendix 2**.
- ❷ Reagent not contained in the kit, use the 10x buffer concentrate supplied with the DNA polymerase of your supplier.
- ❸ Passive reference dye solution only required for usage with ABI PRISM instrument (Applied Genomics/Applied Biosystems)

Table 5: Thermal cycle program (2-step PCR) using ABI PRISM 7000 SDS (Applied Biosystems), for further real-time instruments see **Appendix 1**.

	HOLD	CYCLE	
shut off « 9600 emulation » (window « instruments »)			
Temperature (°C)	95	95	59
Time (min:s)	10:00	00:30	01:30
Cycles		40	

8.2.2. Detection of Internal Control DNA using Rotor-Gene 3000/6000 instrument

Preparation of reagent mixes and samples

1. For preparation of the “5x reagent mix” please refer to **Table 1** or **Table 2**, respectively.

2. Prepare a final reaction mix as shown in **Table 6**. A proposed calculation scheme for the preparation of the reaction mix is given in **Appendix 3**.

3. Add appropriate aliquots of the reaction mix to 0.1 or 0.2 ml optical PCR tubes considering the volume of sample to be added.

Caution! Do not exceed the final volume of 25 µl.

4. After manual or automated dispensing of the reaction mix, add equal aliquots of extracted DNA samples to the tubes. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with an equal volume PCR-grade water.

5. Seal the tubes with the provided caps. **Always wear gloves when handle the tubes and caps!**

6. Select the positions containing non-target controls (“Blank”) and samples (“Unknown”) from the sample type pop-up menu. Choose the dye “JOE/VIC” from the dye layer pop-up menu!

7. Setup the amplification conditions as shown in **Table 7**.

Table 6: Pipetting scheme for the preparation of *Internal Control* reaction mix; calculated to achieve **a final PCR reaction volume of 25 µl in each tube.**

Reagents	<i>Internal Control</i> reaction mix [µl]	
	1 reaction	final conc.
PCR-grade water	❶	-
10x PCR buffer ❷	2.5	1x
MgCl ₂ (50 mM)	2.5	5 mM
5x Reagent mix (containing dNTP, primers and probe)	5.0	1x
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl) ❷	0.3	1.5 U per reaction

- ❶ Volume depends on the intended volume of DNA sample; supplement to achieve **a final volume of 25 µl. Calculation scheme see Appendix 3.**
- ❷ Reagent not contained in the kit, use the 10x buffer concentrate supplied with the DNA polymerase of your supplier.

Table 7: Thermal cycle program (2-step PCR) using Rotor-Gene 3000/6000

	HOLD	CYCLE	
Temperature (°C)	95	95	59
Time (min:s)	10:00	00:15	01:00
Cycles		45	

8.2.3. Detection of Internal Control DNA using LightCycler instrument

Preparation of reagent mixes and samples

1. For preparation of the “4x reagent mix” please refer to **Table 1** or **Table 2**, respectively.

2. Prepare a final reaction mix as shown in **Table 8**. A proposed calculation scheme for the preparation of the reaction mix is given in **Appendix 4**.

- Add appropriate aliquots of the reaction mix to 20 µl capillaries considering the volume of sample to be added.

Caution! Do not exceed the final volume of 20 µl.

- After manual or automated dispensing of the reaction mix, add equal aliquots of extracted DNA samples to the tubes. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with an equal volume PCR-grade water.

- Seal the capillaries with caps. **Always wear gloves when handle the tubes and caps!**

- Select the positions containing non-target controls (“Blank”) and samples (“Unknown”) from the sample type pop-up menu.

- Setup the amplification conditions as shown in **Table 9**.

Table 8: Pipetting scheme for the preparation of *Internal Control* reaction mix; calculated to achieve **a final PCR reaction volume of 20 µl in each capillary.**

Reagents	<i>Internal Control</i> reaction mix [µl]	
	1 reaction	final conc.
PCR-grade water ❶	-	-
10x PCR buffer, LC ❷	2.0	1x
MgCl ₂ (50 mM)	2.0	5 mM
4x Reagent mix (containing dNTP, primers and probe)	5.0	1x
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl) ❸	0.3	1.5 U per reaction

❶ Volume depends on the intended volume of DNA sample; supplement to achieve **a final volume of 20 µl.** Calculation scheme see Appendix 4.

❷ The 10x PCR-buffer, LC, contained in this module, is optimized for using with the AmpliTaq Gold DNA Polymerase (see on page 4). If you use another Taq polymerase, please utilize the corresponding PCR buffer and supplement the reaction mix with 10x BSA solution to a final concentration of 1x (e.g. 2 µl per 20 µl reaction mix).

❸ Reagent not contained in the kit

Table 9: Thermal cycle program using LightCycler 1.0

Program:

Program:	Denaturation			Type:	None	Cycles	1
Segment Number	Temperature Target (°C)	Hold Time (min)	Slope (°C/sec)	2° Target Temp (°C)	Step Size (°C)	Step Delay (Cycles)	Acquisition Mode
1	95	10	20	0	0	0	None

Program:	Amplification			Type:	Quantification	Cycles	50-60
Segment Number	Temperature Target (°C)	Hold Time (sec)	Slope (°C/sec)	2° Target Temp (°C)	Step Size (°C)	Step Delay (Cycles)	Acquisition Mode
1	95	10	20	0	0	0	None
2	59	40	20	0	0	0	Single

Program:	Cooling			Type:	None	Cycles	1
Segment Number	Temperature Target (°C)	Hold Time (sec)	Slope (°C/sec)	2° Target Temp (°C)	Step Size (°C)	Step Delay (Cycles)	Acquisition Mode
1	40	30	20	0	0	0	None

Fluorescence Settings

LED Power	CALIB	Display Mode	3.5 Compatible
Color	N/A		
Compensation	N/A		
Car. Movement	Continuous		

Quantification Settings

Channel Setting	F1
Program Name:	Amplification

8.2.4. Detection of Internal Control DNA using SmartCycler instrument

Preparation of reagent mixes and samples

1. For preparation of the “5x reagent mix” please refer to **Table 1** or **Table 2**, respectively.

2. Prepare a final reaction mix as shown in **Table 10**. A proposed calculation scheme for the preparation of the reaction mix is given in **Appendix 5**.

3. Add appropriate aliquots of the reaction mix to 25 µl optical PCR tubes considering the volume of sample to be added.

Caution! Do not exceed the final volume of 25 µl.

4. After manual or automated dispensing of the reaction mix, add equal aliquots of extracted DNA samples to the tubes. Always run at least one negative control with the samples. To prepare a negative control, replace the template DNA sample with an equal volume PCR-grade water.

5. Seal the tubes with the provided caps. **Always wear gloves when handle the tubes and caps!**

6. Select the positions containing non-target controls (“Blank”) and samples (“Unknown”) from the sample type pop-up menu.
For real-time detection of *Internal Control* DNA the SmartCycler instrument must be calibrated with the dye “VIC/HEX”. The Yakima Yellow labelled *Internal Control* probe has to be measured within this newly calibrated dye channel.

7. Setup the amplification conditions as shown in **Table 11**.

Table 10: Pipetting scheme for the preparation of *Internal Control* reaction mix; calculated to achieve **a final PCR reaction volume of 25 µl in each tube**.

Reagents	<i>Internal Control</i> reaction mix [µl]	
	1 reaction	final conc.
PCR-grade water	①	-
10x PCR buffer, LC ②	2.5	1x
MgCl ₂ (50 mM)	2.5	5 mM
5x Reagent mix (containing dNTP, primers and probe)	5.0	1x
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl) ③	0.3	1.5 U per reaction

- ① Volume depends on the intended volume of DNA sample; supplement to achieve **a final volume of 25 µl**. Calculation scheme see Appendix 5.
- ② The 10x PCR-buffer, LC, contained in this module, is optimized for using with the AmpliTaq Gold DNA Polymerase (see on page 4). If you use another Taq polymerase, please utilize the corresponding PCR buffer and supplement the reaction mix with 10x BSA solution to a final concentration of 1x (e.g. 2.5 µl per 25 µl reaction mix).
- ③ Reagent not contained in the kit

Table 11: Thermal cycle program using SmartCycler instrument

	Hold	Cycle	
Temperature (°C)	95	95	59
Time (min:s)	10:00	00:15	00:30
Cycles		50	
Ramping		2.5 °C/sec	

8.3. Interpretation of data

Please follow the recommendations of the manufacturer of the real-time instrument to determine the C_T values of the samples.

All samples with equal DNA extraction efficiency should have similar C_T values. Depending on the DNA extraction method the absolute value of the mean C_T can vary.

9. Stability and precision, quality assessment*

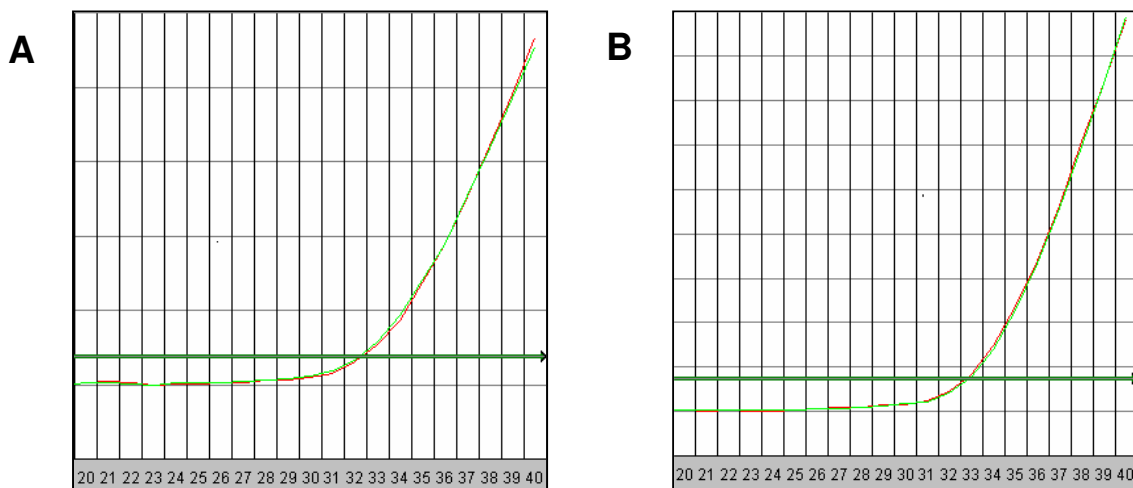


Figure 1: Saturation curves obtained from co-purified Internal Controls. Tubes containing Internal Controls (10.000 copies) stored for one week at either -20°C (A) or room temperature (B) were extracted with the AJ Roboscreen RTP® DNA/RNA Virus Supersense Kit in the presence of 1 ml of control serum. 5 μl of purified DNA sample were amplified in duplicate by a standard real-time PCR protocol using an ABI PRISM 7000 SDS. The mean C_T values were 32.26 ± 0.06 (A) and 32.85 ± 0.06 (B), respectively, indicating excellent stability even at room temperature. The average recovery rate was 30%.

* determined according to the ICH-Guidelines "Validation of analytical methods: Methodology", ICH Topic Q2B

10. Carry-over prevention using UNG (optional)

The heat-labile Uracil-DNA Glycosylase (UNG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions and the pretreatment of all successive PCR mixtures with the UNG. The UNG enzyme cleaves DNA at 37°C at any site where a dUTP residue has been incorporated. Subsequently, the resulting abasic sites are hydrolyzed due to high temperatures during the initial denaturation step, and can not serve as PCR template any longer. The heat-labile UNG is inactivated at the same time. We recommend the application of 0.2 U UDG per assay with an initial 15-min incubation step at 37°C prior to amplification³.

Warranty Notice

During the warranty period the AJ Roboscreen ready-to-use nucleic acid Internal Controls allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. The purchase of this product does not convey any right for its use in clinical diagnostic applications.

References

1. Köhler T, Lerche D, Meye A, Weisbrich C, Wagner O. Automated analysis of nucleic acids by quantitative PCR using DNA coated ready-to-use reaction tubes. *J.Lab.Med.* 1999; 23:408-414.
2. Köhler T, Schill C, Deininger MW, Krahl R, Borchert S, Hasenclever D, Leiblein S, Wagner O. High Bad and Bax mRNA expression correlate with negative outcome in acute myeloid leukemia (AML). *Leukemia* 2002; 6: 22-29.
3. Köhler T, Rost AK, Remke H. Calibration and storage of DNA competitors used for contamination-protected competitive PCR. *Biotechniques* 1997; 23:722-726.

Appendix 1: Recommended amplification conditions for different real-time instruments

• **ABI PRISM 7700 SDS (Applied Biosystems)**

	HOLD	CYCLE	
Temperature (°C)	95	95	59
Time (min:s)	10:00	00:15	01:00
Cycles		40	

➔ Seal the tubes with optical caps or appropriate plastic film e.g. ABI PRISM Optical Adhesive Covers (Applied Biosystems, Cat.No.: 4311971)*.

• **iCycler IQ (BioRad)**

	HOLD	CYCLE	
setting the ramping rate: 2.5 °C per sec			
Temperature (°C)	95	95	59
Time (min:s)	10:00	00:20	01:00
Cycles		40	

➔ Seal the tubes with optical caps or appropriate plastic film e.g. ABI PRISM Optical Adhesive Covers (Applied Biosystems, Cat.No.: 4311971)*.

*) How to use the film:

1. Tailor the cover according to the number of used wells.
2. To avoid leakage during amplification, after placing on the wells tightly press on the cover and use a compression pad (Applied Biosystems, Cat.No.: 4312639), follow the instructions of the manufacturer!

Appendix 2: Calculation scheme for the preparation of an *Internal Control* reaction mix for use with ABI PRISM 7700/7000 SDS; iCycler IQ

Calculation example: expected volume of DNA sample is 5 µl.

Preparation of 20 µl reaction mix to add to the DNA sample in order to achieve a final PCR reaction volume of 25 µl.

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for 100 reactions
DNA sample volume	5.0 µl	
Volume of reaction mix for a final volume of 25 µl	20.0 µl	
PCR grade water	7.2 µl	720 µl
10x PCR buffer	2.5 µl	250 µl
10x Passive reference dye solution	2.5 µl	250 µl
MgCl ₂ (50 mM)	2.5 µl	250 µl
5x Reagent mix	5.0 µl	500 µl
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl	30 µl

For your individual calculations:

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for x reactions	Mastermix for x reactions
DNA sample volume			
Volume of mastermix			
PCR grade water			
10x PCR buffer	2.5 µl		
10x Passive reference dye solution	2.5 µl		
MgCl ₂ (50 mM)	2.5 µl		
5x Reagent mix	5.0 µl		
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl		

Appendix 3: Calculation scheme for the preparation of an *Internal Control* reaction mix for use with Rotor-Gene 3000/6000

Calculation example: expected volume of DNA sample is 5 µl.

Preparation of 20 µl reaction mix to add to the DNA sample in order to achieve a final PCR reaction volume of 25 µl.

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for 80 reactions
DNA sample volume	5.0 µl	
Volume of reaction mix for a final volume of 25 µl	20.0 µl	
PCR grade water	9.7 µl	776 µl
10x PCR buffer	2.5 µl	200 µl
MgCl ₂ (50 mM)	2.5 µl	200 µl
5x Reagent mix	5.0 µl	400 µl
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl	24 µl

For your individual calculations:

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for x reactions	Mastermix for x reactions
DNA sample volume			
Volume of mastermix			
PCR grade water			
10x PCR buffer	2.5 µl		
MgCl ₂ (50 mM)	2.5 µl		
5x Reagent mix	5.0 µl		
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl		

Appendix 4: Calculation scheme for the preparation of an *Internal Control* reaction mix for use with LightCycler

Calculation example: expected volume of DNA sample is 5 µl.

Preparation of 15 µl reaction mix to add to the DNA sample in order to achieve a final PCR reaction volume of 20 µl.

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for 35 reactions
DNA sample volume	5.0 µl	
Volume of reaction mix for a final volume of 25 µl	15.0 µl	
PCR grade water	5.7 µl	199.5 µl
10x PCR buffer, LC	2.0 µl	70 µl
MgCl ₂ (50 mM)	2.0 µl	70 µl
4x Reagent mix	5.0 µl	175 µl
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl	10.5 µl

For your individual calculations:

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for x reactions	Mastermix for x reactions
DNA sample volume			
Volume of mastermix			
PCR grade water			
10x PCR buffer, LC	2.0 µl		
MgCl ₂ (50 mM)	2.0 µl		
4x Reagent mix	5.0 µl		
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl		

Appendix 5: Calculation scheme for the preparation of an *Internal Control* reaction mix for use with SmartCycler

Calculation example: expected volume of DNA sample is 5 µl.

Preparation of 20 µl reaction mix to add to the DNA sample in order to achieve a final PCR reaction volume of 25 µl.

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for 18 reactions
DNA sample volume	5.0 µl	
Volume of reaction mix for a final volume of 25 µl	20.0 µl	
PCR grade water	9.7 µl	174.6 µl
10x PCR buffer, LC	2.5 µl	45 µl
MgCl ₂ (50 mM)	2.5 µl	45 µl
5x Reagent mix	5.0 µl	90 µl
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl	5.4 µl

For your individual calculations:

Reagents	<i>Internal Control</i> reaction mix for 1 reaction	Mastermix for x reactions	Mastermix for x reactions
DNA sample volume			
Volume of mastermix			
PCR grade water			
10x PCR buffer, LC	2.5 µl		
MgCl ₂ (50 mM)	2.5 µl		
5x Reagent mix	5.0 µl		
Taq polymerase (e.g. AmpliTaq Gold) (5 U/µl)	0.3 µl		

Your notes



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