Biometra

PRODUCT LINE

analytikjena

Demofiles qPCRsoft

Absolute Quantification

Relative Quantification

ΔΔCt Quantification

Melting curve

Genotyping

Multigene-/Multiplate-Analysis

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Analysis
Absolute Quantification

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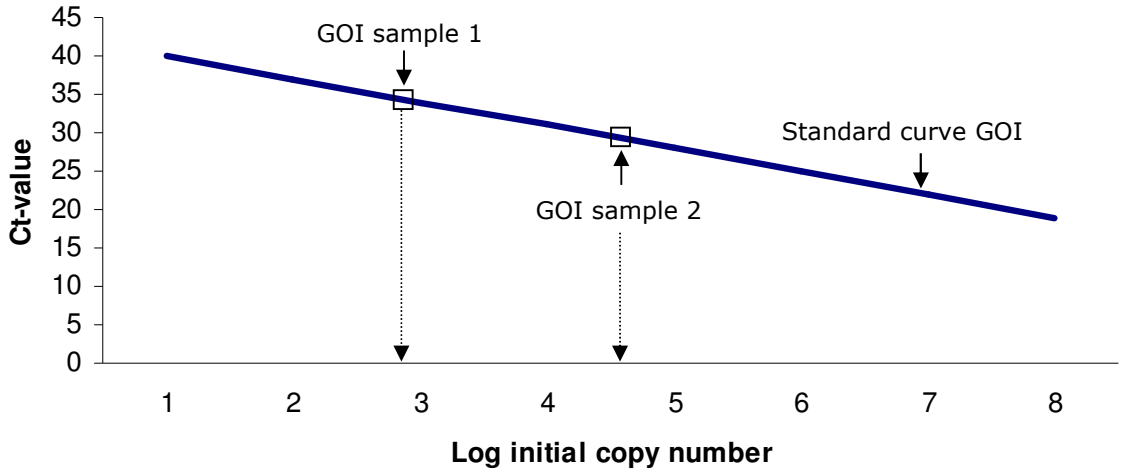
- Goal: Determination of absolute copy number of nucleic acids for a sample
- 1. Measure a standard curve for the GOI with defined concentrations
- 2. Measure the Ct value for the GOI in a sample and determine the copy number



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Analysis
Absolute Quantification

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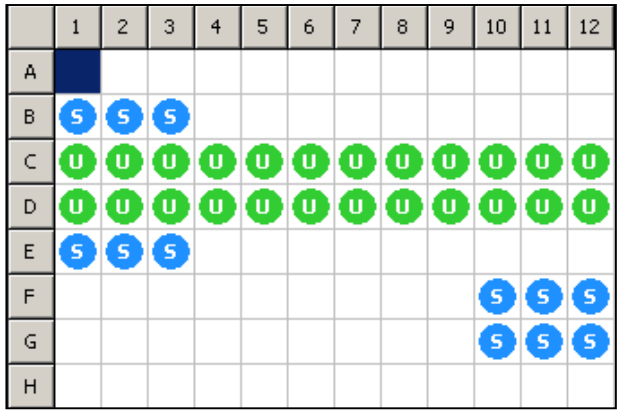
- 1 standard, 4 concentrations, 3 replicates each
  - 1000ng (Well E1-E3)
  - 100ng (Well B1-B3)
  - 10ng (Well G10-12)
  - 1ng (Well F10-F12)
- 8 samples of unknown concentration (Well C1-D12), 3 replicates each



- All samples measured with FAM

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Analysis
Absolute Quantification

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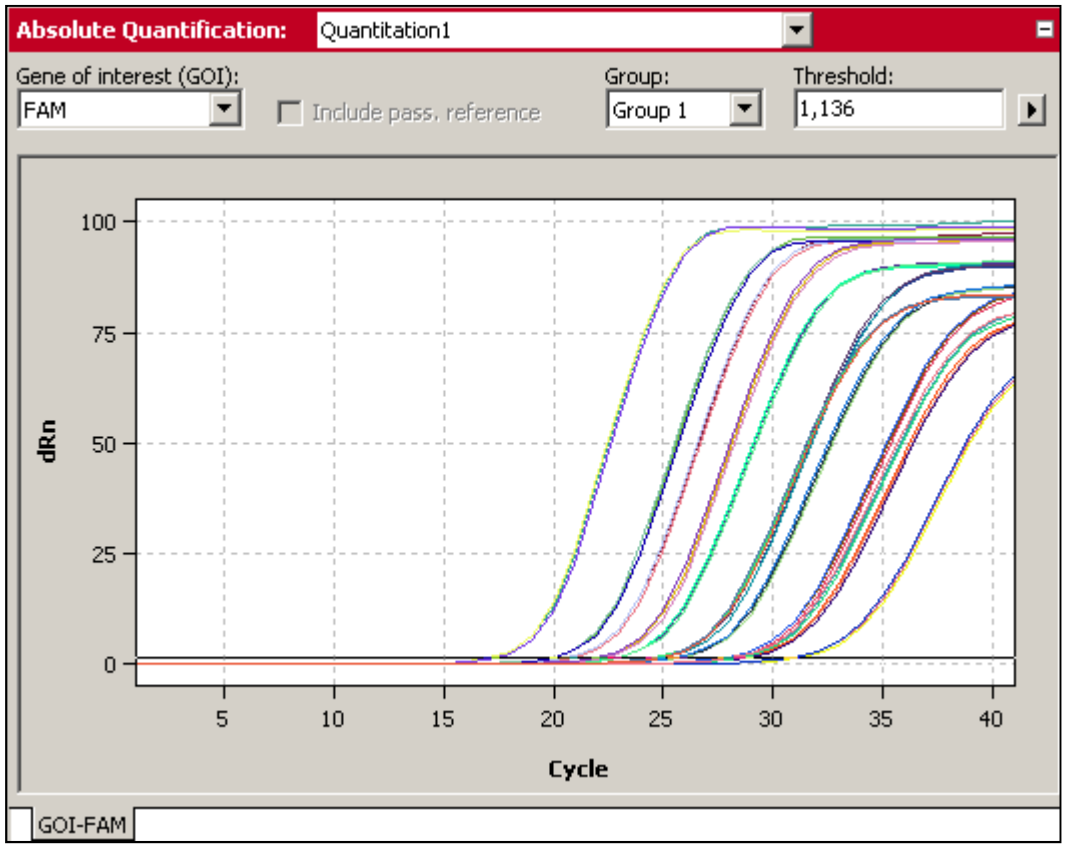
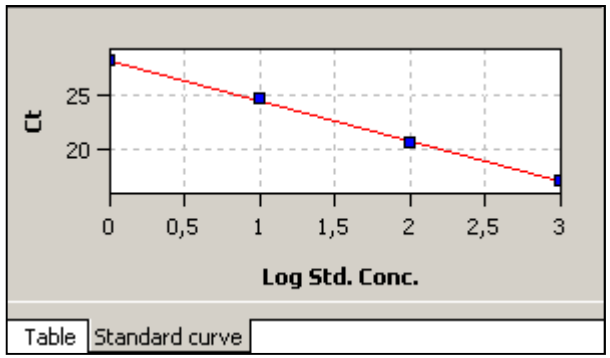
- Amplification curves for the samples measured with FAM are shown



Analysis
Absolute Quantification

- For the standard the log concentration is plotted against Ct-values



- By the mean Ct-values of the samples the concentration is calculated

Well		Sample name	Sample type	Group	Ct	Mean Ct	Conc. Std.	Mean Conc.
D9		sample7	Unknown	Group 1	27,6	27,68		1,2994
D10		sample8	Unknown	Group 1	30,65	30,69		0,1992
D11		sample8	Unknown	Group 1	30,87	30,69		0,1992
D12		sample8	Unknown	Group 1	30,55	30,69		0,1992
E1		Std1	Standard	Group 1	17,03	17,05	1000	958,8402
E2		Std1	Standard	Group 1	17	17,05	1000	958,8402
E3		Std1	Standard	Group 1	17,12	17,05	1000	958,8402

Table | Standard curve

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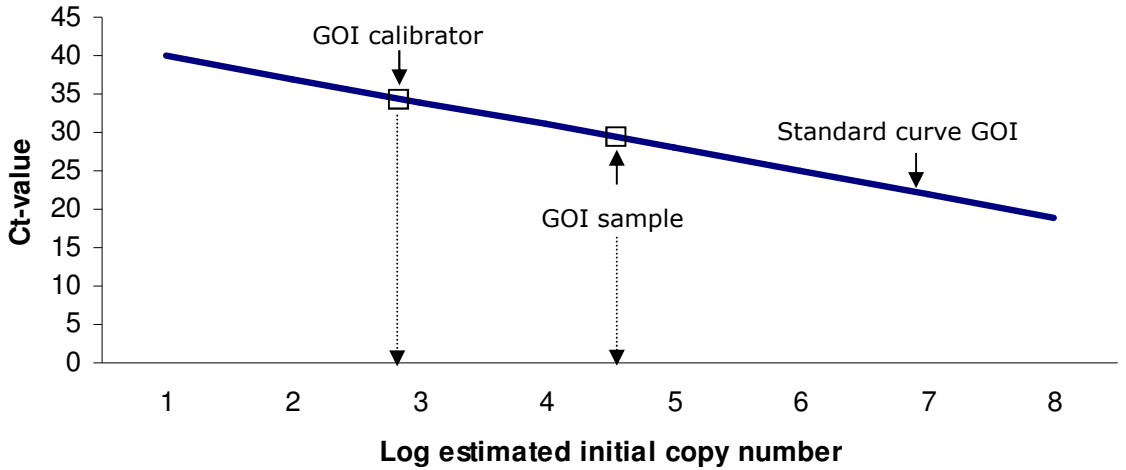
Analysis
Relative Quantification

analytikjena

- Goal: Determination of relative expression rates
- 1. Measure a standard curve for the GOI with defined dilutions
- 2. Measure the Ct value for the GOI in a sample and a calibrator and calculate the relative expression rate



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Analysis
Relative Quantification

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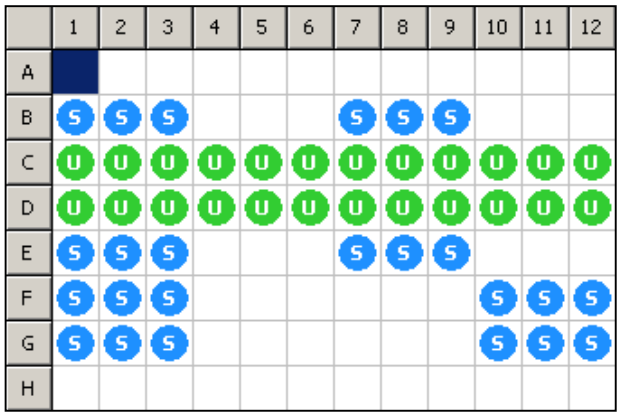
- 2 standards, 4 concentrations, 3 replicates each

	c-myc	GAPDH
▪	10000ng (Well G1-G3)	15000ng (Well E1-E3)
▪	1000ng (Well F1-F3)	1500ng (Well B1-B3)
▪	100ng (Well E7-E9)	150ng (Well G10-G12)
▪	10ng (Well B7-B9)	15ng (Well F10-F12)

- 8 samples of unknown concentration (Well C1-D12), 3 replicates each



- All samples measured with FAM

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Analysis
Relative Quantification

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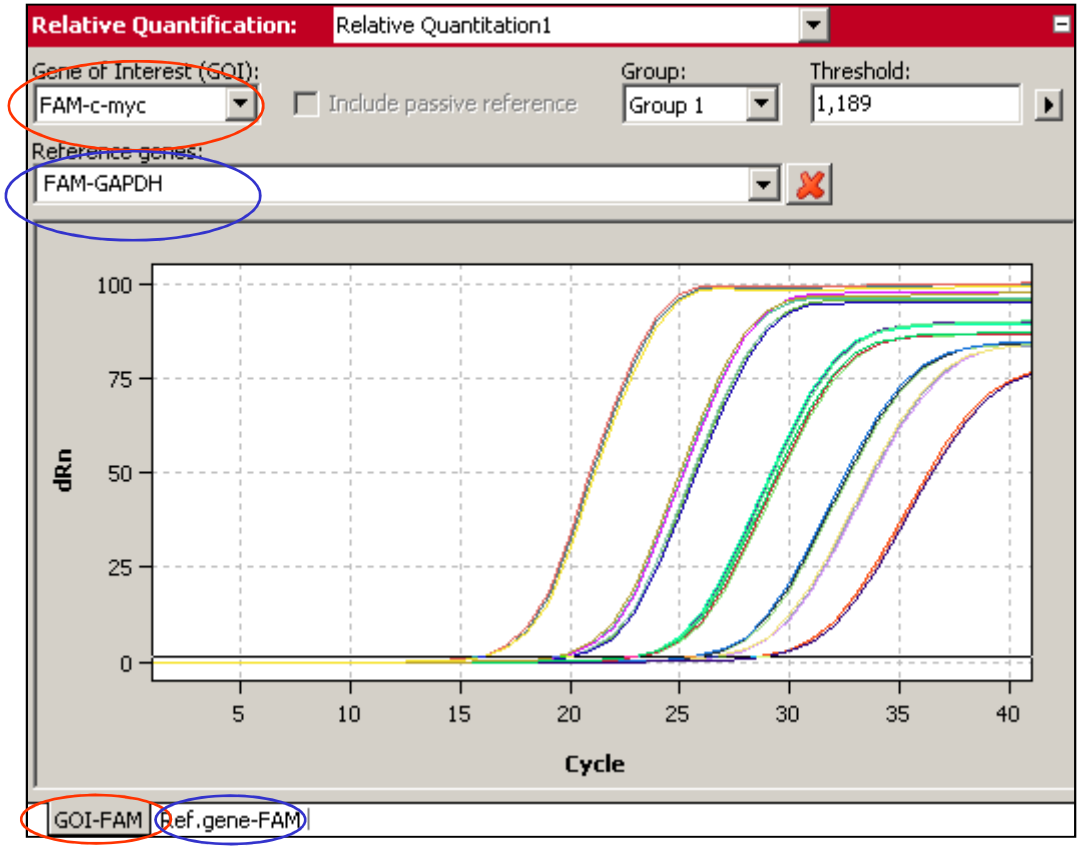
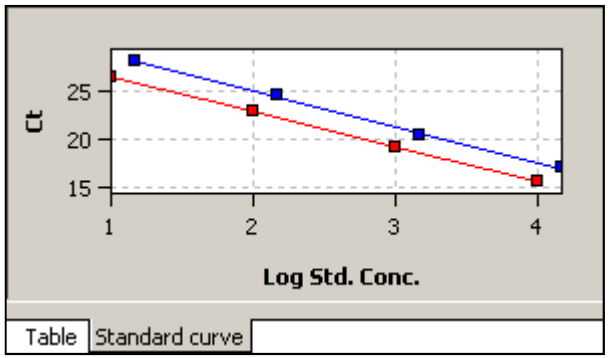
- Amplification curves for the GOI and reference genes measured with the selected dyes are shown



Analysis
Relative Quantification

- For the standards the log concentration is plotted against Ct-values



- By the mean Ct-values of the samples the relative concentration is calculated

Well		Sample name	Group	GOI	Reference gene	Ct GOI	Ct Reference ge	Mean Ct GOI	Mean Ct Ref.ge	Relative Conc.
C9		Sample3	Group 1	c-myc	c-myc	25,38		25,52		0,9655
C10		Sample4	Group 1	c-myc	c-myc	28,43		28,5		0,9306
C11		Sample4	Group 1	c-myc	c-myc	28,45		28,5		0,9306
C12		Sample4	Group 1	c-myc	c-myc	28,64		28,5		0,9306
D1		Sample1	Group 1	GAPDH	GAPDH		21,99		21,99	1,0482
D2		Sample1	Group 1	GAPDH	GAPDH		22,07		21,99	1,0482
D3		Sample1	Group 1	GAPDH	GAPDH		21,91		21,99	1,0482

Table | Standard curve

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Analysis
ΔΔCt-Method

analytikjena

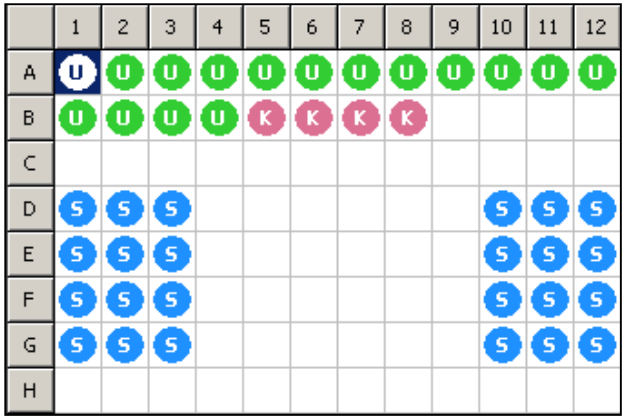
- Goal: Determination of relative expression rates
- 1. Measure the Ct values for the GOI in a sample and a calibrator
- 2. Measure the Ct values for the reference gene (RefGen) in a sample and a calibrator
- 3. Calculate the relative expression rate by the ΔΔCt-Method
  - Sample: ΔCt = Ct (GOI) – Ct (RefGen)
  - Calibrator: ΔCt = Ct (GOI) – Ct (RefGen)
    - ΔΔCt = ΔCt (Sample) - ΔCt (Calibrator)
    - $2^{-\Delta\Delta Ct} \rightarrow$ n-fold expression sample to calibrator

Analysis
ΔΔCt-Method

- GOI = c-myc, RefGen = GAPDH
- 1 calibrator GOI (Well B5-B6) and RefGen (Well B7-B7), 2 replicates each
- 4 samples (Well A1-B4) GOI and RefGen, 2 replicates each
- 2 standards, 4 dilutions, 3 replicates each

c-myc	GAPDH
0.001x (Well D1-D3)	0.001x (Well D10-D12)
0.01x (Well E1-E3)	0.01x (Well E10-E12)
0.1x (Well F1-F3)	0.1x (Well F10-F12)
1x (Well G1-G3)	1x (Well G10-G12)

- All samples measured with FAM



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Analysis
ΔΔCt-Method

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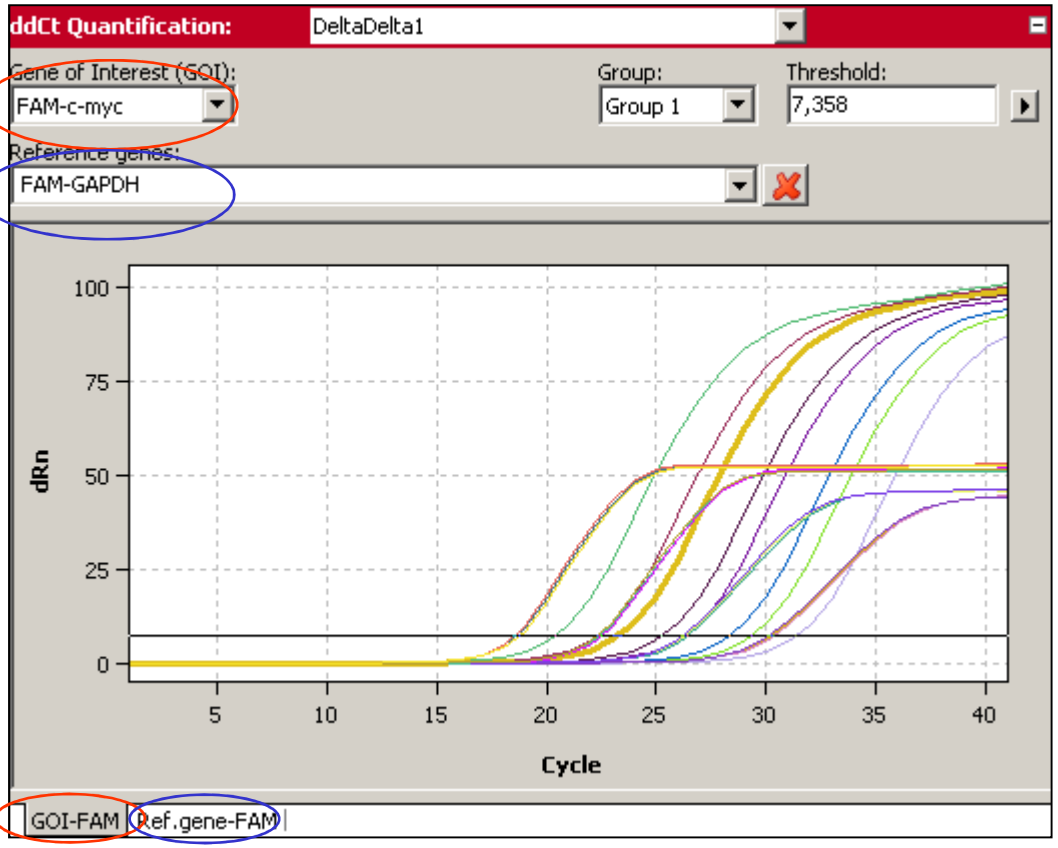
- Amplification curves for the GOI and reference genes measured with the selected dyes are shown



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Analysis
ΔΔCt-Method

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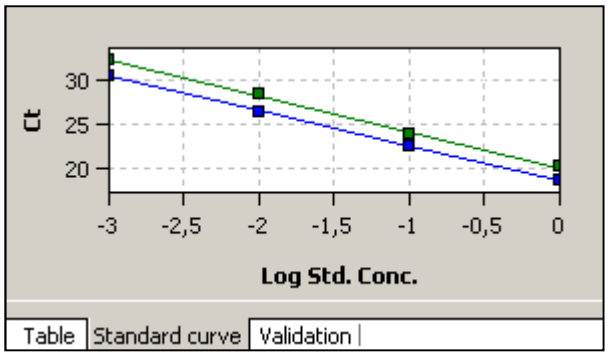
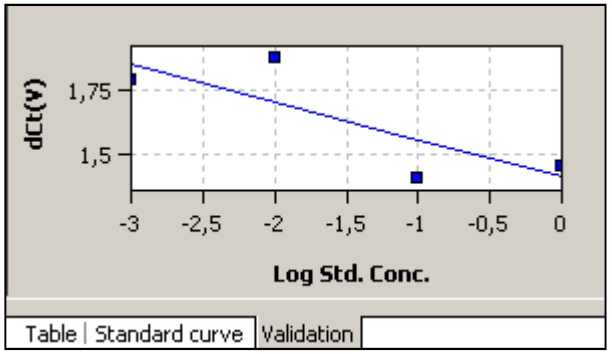
- For the standards the log concentration is plotted against Ct-values. Additionally, a validation curve is calculated to compare the efficiency of the amplification of the GOI and RefGen.





- By the dCt-values the normalised expression ratio is calculated

Well		Sample name	Group	GOI	Reference gene	Mean Ct GOI	Mean Ct Ref.ge	dCt GOI	dCt Ref.gene	Norm. Expressio
A1		Sa1	Group 1	c-myc	c-myc	24,32		-2,96		0,1286
A2		Sa1	Group 1	c-myc	c-myc	24,32		-2,96		0,1286
A3		Sa1	Group 1	GAPDH	GAPDH		19,41		0	0,1286
A4		Sa1	Group 1	GAPDH	GAPDH		19,41		0	0,1286
A5		Sa2	Group 1	c-myc	c-myc	30,39		-9,03		0,001
A6		Sa2	Group 1	c-myc	c-myc	30,39		-9,03		0,001
A7		Sa2	Group 1	GAPDH	GAPDH		18,43		0,98	0,001

Table | Standard curve | Validation

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Analysis
Melting curve

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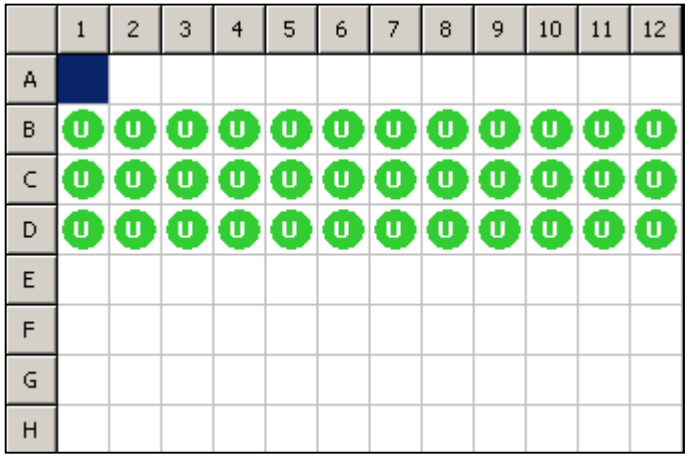
- Goal: Determination of the melting temperature of PCR products
- 1. Increase the temperature in small steps
- 2. Calculate the first derivate of the melting curve
- 3. The peak defines the melting temperature Tm

- 8 unknown samples measured with SYBR-Green, 3 replicates each



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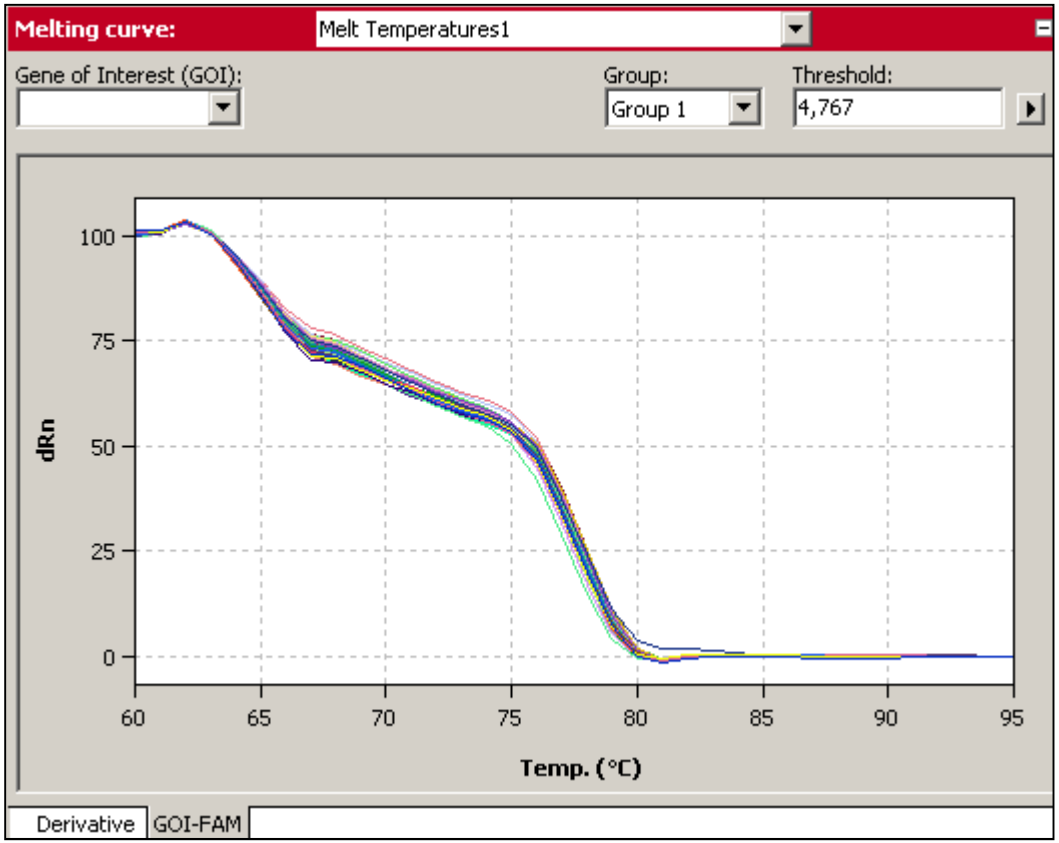
Analysis
Melting curve

analytikjena

- Melting curves for the samples are shown



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Analysis
Melting curve

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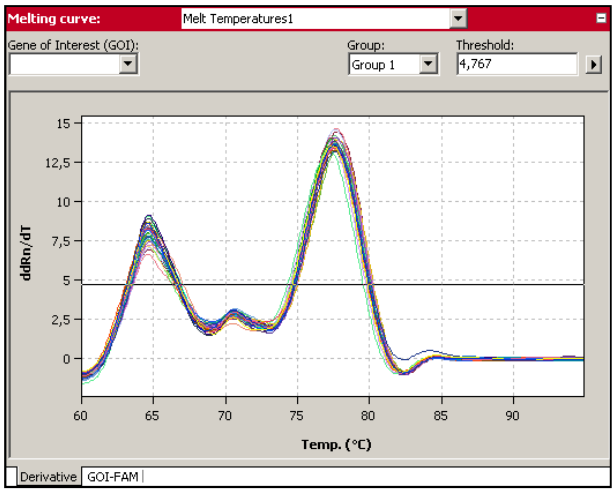
- The derivative of the melting curve is calculated



- The highest peak gives the Tm value

Well		Sample name	Sample type	Group	Tm	Mean Tm
B1		Smp1	Unknown	Group 1	77,8	77,7
B2		Smp1	Unknown	Group 1	77,7	77,7
B3		Smp1	Unknown	Group 1	77,6	77,7
B4		Smp2	Unknown	Group 1	77,6	77,5
B5		Smp2	Unknown	Group 1	77,5	77,5
B6		Smp2	Unknown	Group 1	77,4	77,5
B7		Smp3	Unknown	Group 1	77,4	77,4

Table

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Analysis
Genotyping

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- Goal: Determining the genetic make-up (genotype) of an individual
- Analysis of single base substitutions (SNP-analysis)
- 2 different labeled probes are used
- One probe only binds to the wild-type sequence, the second one to the mutated sequence
- Example:
- 5`- AGTGTCATCGTACGTACGTGTTAC -3` FAM-labeled probe (wild-type)
- 5`- AGTGTCATCGTATGTACGTGTTAC -3` ROX-labeled probe (mutant)
- The fluorescence signal of both probes is measured to determine the genotype of the sample

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Analysis
Genotyping

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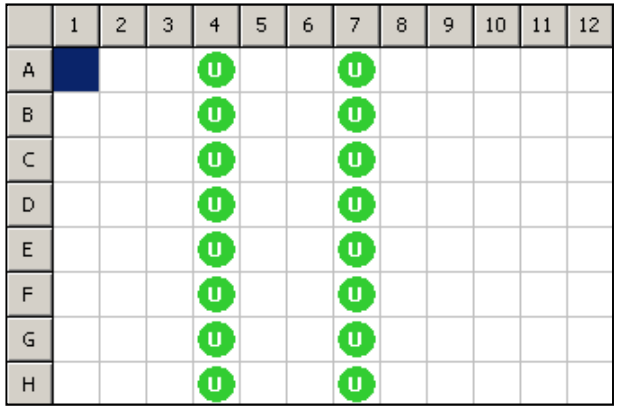
- 16 samples (6 x 2 replicates and 2 single samples)
  - Sample H1 (Well A4-B4)
  - Sample H2 (Well C4-D4)
  - Sample H3 (Well E4-F4)
  - Sample M1 (Well G4-H4)
  - Sample WT1 (Well A7-B7)
  - Sample WT2 (Well C7-D7)
  - Sample WT3 (Well E7)
  - Sample M2 (Well F7-G7)
  - Sample M3 (Well G8)



- All samples measured with FAM and ROX

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Analysis
Genotyping

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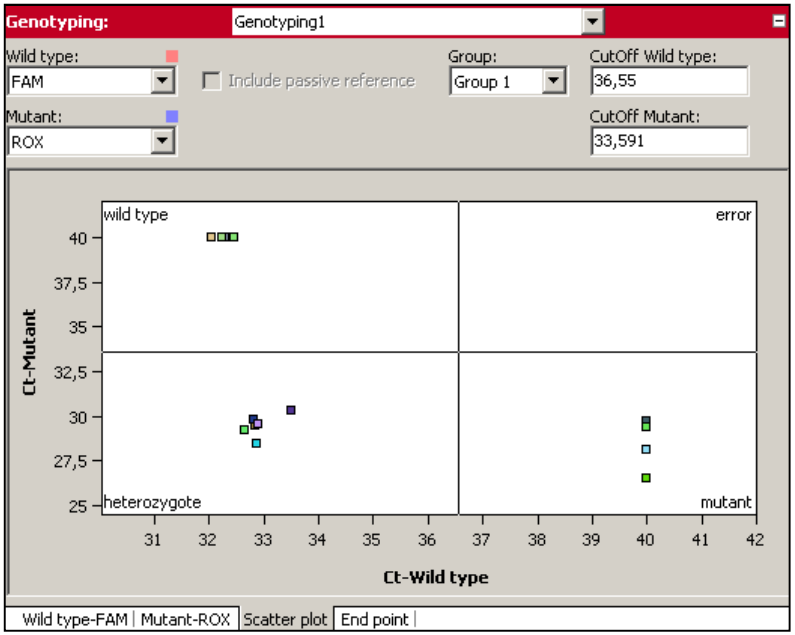
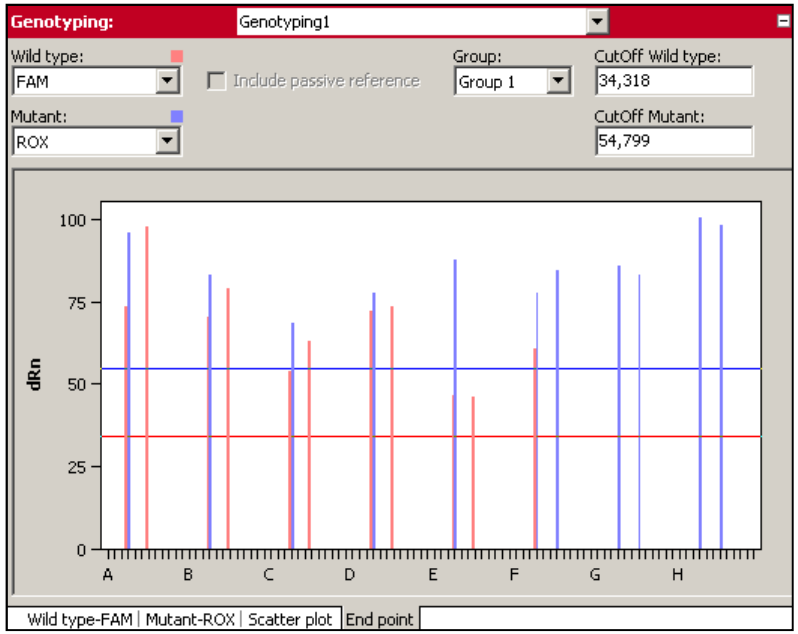
- Results are shown as scatter-plot or bar chart





- The genotype can be wild-type (FAM), mutant (ROX), heterozygous (FAM + ROX)
- The vertical and horizontal line in the scatter-plot and the two horizontal lines in the bar chart can be moved to change the cut-off value

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Analysis
Genotyping

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- The genotype of the sample is also listed in the results table

Well		Sample name	Sample type	Group	Genotyp	Reaction Wild ty	Reaction Mutanl	Genotyp Replica
A3			Empty	Group 1				
A4		H1	Unknown	Group 1	heterozygote	yes	yes	heterozygote
A5			Empty	Group 1				
A6			Empty	Group 1				
A7		WT1	Unknown	Group 1	wild type	yes	no	wild type
A8			Empty	Group 1				
A9			Empty	Group 1				

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Analysis
Multigene-/Multiplate-Analysis

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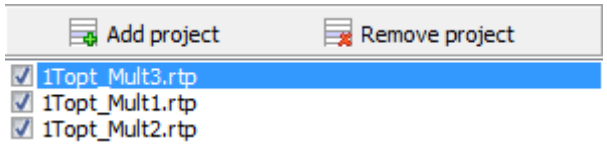
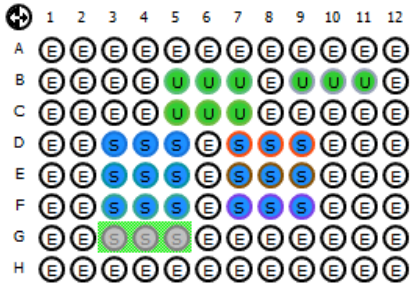
Multiplex Multigene-/Multiplate Analysis

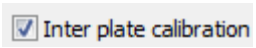
- Goal: Analyse multiple genes or results from multiple plates

1. Perform real-time PCR experiments inclusive ΔΔCt-analysis
2. Open Multigene-/Multiplate-Analysis 
3. Add project files



4. Assign Interplate-Standards (IPS)



5. Activate inter plate calibration 

6. Calculate results

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Analysis
Multigene-/Multiplate-Analysis

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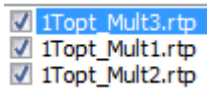
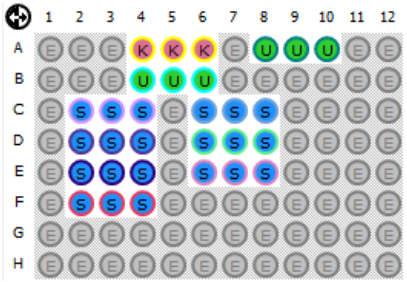
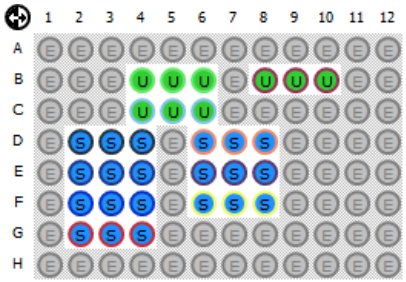
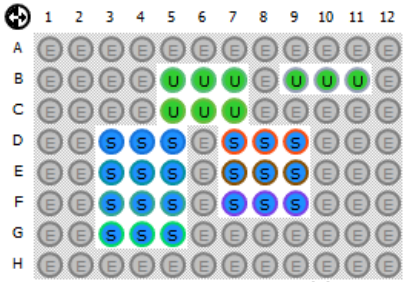
- 3 project files
  - 1TOpt_Multi1.rtp
  - 1TOpt_Multi2.rtp
  - 1TOpt_Multi3.rtp



- 4 genes measured with 4 dyes
  - FAM-Tubulin
  - VIC-Actin
  - ROX-IL_1b
  - Cy5-GAPDH
- Samples measured as triplicates
- Slightly different plate layouts:


1TOpt_Multi1.rtp


1TOpt_Multi2.rtp


1TOpt_Multi3.rtp

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Analysis
Multigene-/Multiplate-Analysis

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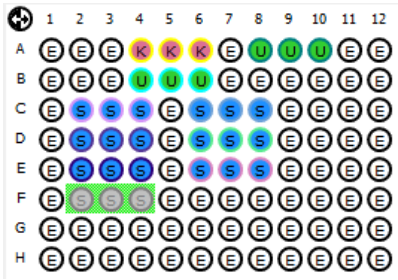
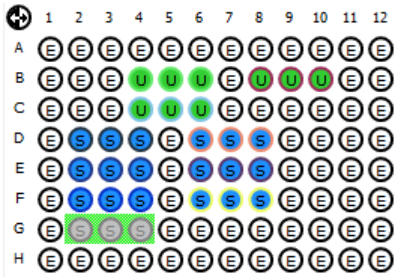
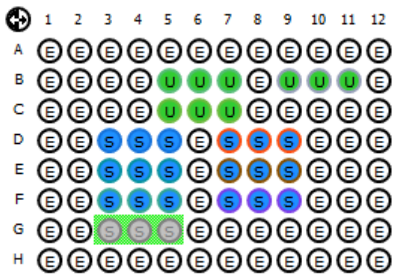
- Interplate Standards (IPS):
  - 1TOpt_Multi1.rtp F2-F4
  - 1TOpt_Multi2.rtp G2-G4
  - 1TOpt_Multi3.rtp G3-G5



1TOpt_Multi1.rtp

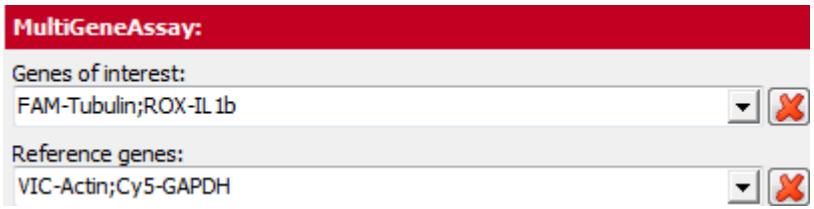


1TOpt_Multi2.rtp



1TOpt_Multi3.rtp

- Genes of Interest (GOI) FAM-Tubulin and ROX-IL 1b
- Reference Genes VIC-Actin and Cy5-GAPDH



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Analysis
Multigene-/Multiplate-Analysis

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- If the ☑ Inter plate calibration is set active a correction value is calculated from the IPS

Correction calculation:

$$Ct_{i,p}^{corr} = Ct_{i,p}^{meas} - \overline{Ct}_{p}^{IPC} + \frac{1}{N}\sum_{p=1}^{N} Ct_{p}^{IPC}$$

with

$Ct_{i,p}^{corr}$ – corrected Ct – value for replicate i on plate p
$Ct_{i,p}^{meas}$ – measured Ct – value for replicate i on plate p
$\overline{Ct}_{p}^{IPC}$ – mean value of Ct – values of IPS – samples on plate p
$\frac{1}{N}\sum_{p=1}^{N} Ct_{p}^{IPC}$ – mean value of Ct – values of all IPS – samples on all N plates

- The correction values are summarized on the tab "IPS"

Project name	Dye	Mean Ct (IPS, Project)	Mean Ct (IPS, all Projects)	Correction value
1Topt_Mult3.rtp	FAM	32,01	31,71	-0,3
1Topt_Mult3.rtp	VIC	33,15	31,95	-1,2
1Topt_Mult3.rtp	ROX	31,11	30,46	-0,65
1Topt_Mult3.rtp	Cy5	30,56	29,78	-0,78
1Topt_Mult1.rtp	FAM	30,71	31,71	1,01
1Topt_Mult1.rtp	VIC	29,79	31,95	2,15

Table | IPS

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Analysis

Multigene-/Multiplate-Analysis

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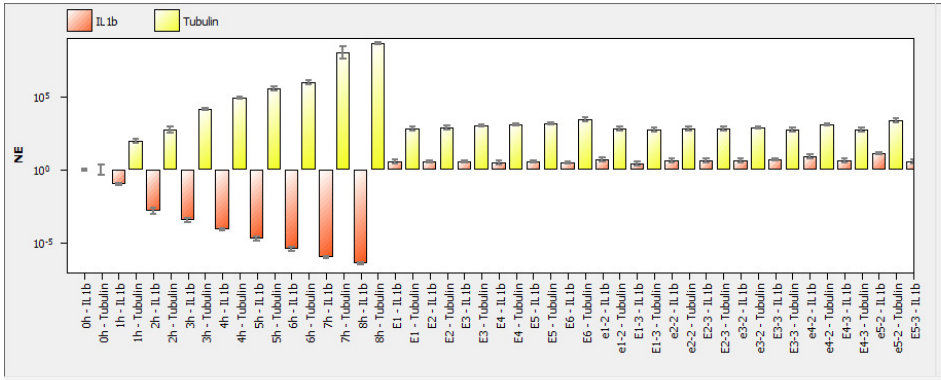
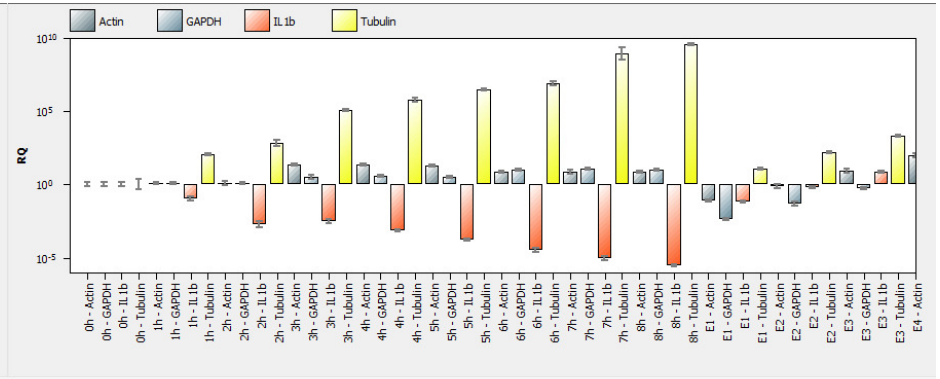
- The software automatically calculates the results and draws bar charts for:
  - Normalized expression
  - Relative quantity
- The calculated values are summarized in the results table

Normalized expression



Relative quantity



Normalized expression | Relative quantity

Project name	Gene	Sample name	No. of repl...	Mean Ct	Mean calib...	Std.Dev. ...	RQ	Std. Dev. ...	Norm. exp...	Std.Dev.n...
1Topt_Mult3.rtp	Tubulin-FAM	7h	3	2,02	1,72	3,5	741957710,41	1799311274,81	90606047,15	219758271,45
1Topt_Mult1.rtp	Tubulin-FAM	0h	3	30,18	31,19	2,39	1	1,65	1	1,65
1Topt_Mult1.rtp	Tubulin-FAM	2h	3	21,04	22,04	1,1	567,64	433,71	512,22	391,68
1Topt_Mult1.rtp	IL 1b-ROX	2h	3	30,31	32,01	1,07	1,64E-3	1,22E-3	1,48E-3	1,10E-3
1Topt_Mult2.rtp	IL 1b-ROX	e6-2	3	9,85	8,79	0,56	16083,34	6274,74	15,46	6,56
1Topt_Mult3.rtp	[Actin-VIC]	E5-3	3	15,61	14,4	0,51	1117,43	398,12		

Table | IPS

Normalized expression | Relative quantity

Project name	Gene	Sample name	No. of repl...	Mean Ct	Mean calib...	Std.Dev. ...	RQ	Std. Dev. ...	Norm. exp...	Std.Dev.n...
1Topt_Mult3.rtp	Tubulin-FAM	7h	3	2,02	1,72	3,5	741957710,41	1799311274,81	90606047,15	219758271,45
1Topt_Mult1.rtp	Tubulin-FAM	0h	3	30,18	31,19	2,39	1	1,65	1	1,65
1Topt_Mult1.rtp	Tubulin-FAM	2h	3	21,04	22,04	1,1	567,64	433,71	512,22	391,68
1Topt_Mult1.rtp	IL 1b-ROX	2h	3	30,31	32,01	1,07	1,64E-3	1,22E-3	1,48E-3	1,10E-3
1Topt_Mult2.rtp	IL 1b-ROX	e6-2	3	9,85	8,79	0,56	16083,34	6274,74	15,46	6,56
1Topt_Mult3.rtp	[Actin-VIC]	E5-3	3	15,61	14,4	0,51	1117,43	398,12		

Table | IPS