



# 5x Multiplex QPCR Mix (ROX)

## Data sheet

Order No. BS 76.515.0200	200 µl
Order No. BS 76.515.1000	1,000 µl
Order No. BS 76.515.5000	5,000 µl

(For research and *in vitro* use only)

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For:

Batch No.:

Best before:

Appearance: clear liquid

Colour: Light pink

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<b>Bio&amp;SELL</b>	E-Mail: <a href="mailto:info@bio-sell.de">info@bio-sell.de</a> Internet: <a href="http://www.bio-sell.de">www.bio-sell.de</a>	Fon : +49 (0) 9128 – 724 32 32 Fax : +49 (0) 9128 – 724 32 33
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Lohweg 27  
90537 Feucht bei Nürnberg



## 5x Multiplex QPCR Mix (ROX)

### Description

Bio&SELL 5x QPCR Mix (ROX) is optimized for real-time applications of quantitative multiplex PCR and contains all the necessary components for quantitative PCR except template DNA, primer and probe. Bio&SELL 5x QPCR-Mix (ROX) contains optimized components as well as Bio&SELL Hot-Start Taq-DNA-Polymerase in a proprietary buffer system, which also allows the detection of “Low-Copy-Number-Targets”.

### Composition of the Bio&SELL 5x QPCR Mixes

- Bio&SELL Hot-Start Taq-DNA-Polymerase
- 5x Multiplex QPCR reaction buffer
- 15 mM MgCl<sub>2</sub>, (= 3 mM in 1x solution)
- dATP, dGTP, dCCT and dTTP in an optimized composition (the use of dTTP allows increased reaction sensitivity and efficiency compared to dUTP)
- ROX-dye

### Applications

- Detection and quantification of DNA and cDNA targets in multiplexes
- Profiling of gene expressions
- Microbial detection as duplex / multiplex with internal control
- Determination of viral loads

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## 5x Multiplex QPCR Mix (ROX)

### Cycler and probe compatibility

Bio & SELL 5x Multiplex QPCR Mix (ROX) is compatible with every probe system as well as with real-time cyclers that need ROX as an internal reference:

- ABI PRISM® 5700, 7000, 7300, 7500, 7700, 7900, 7900HT (including Fast-Block)
- StepOne™, StepOne™ Plus
- Stratagene Mx3000PTM, Mx3005PTM und Mx4000®

### ROX-dye:

ROX is a passive, internal reference dye that is used to normalize the fluorescence reporter signal during QPCR.

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## 5x Multiplex QPCR Mix (ROX)

### Recommended pipetting protocol

The individual components are to be pipetted in the following order (fill up to 20 or 25  $\mu$ l final volume):

Component	Volume	Final concentration/ quantity
Bio&SELL 5x Multiplex QPCR Mix (ROX)	4 $\mu$ l	1x
Primer-Mix	x $\mu$ l	200 – 400 nM
Probe-Mix	x $\mu$ l	100 – 250 nM
MgCl <sub>2</sub>	0 - 3 $\mu$ l	3 mM to 6 mM
Template-DNA	x $\mu$ l	1 – 50 ng/ $\mu$ l
Water	Fill up to 20 or 25 $\mu$ l	

Unless they are standard approaches, the primer and probe mix must be titrated for optimal results.

Standard approaches with 3 primer pairs should be set to 200 nM per primer and with 3 probes to 100 nM per probe.

For batches with more than 3 primer pairs and more than 3 probes, higher magnesium concentrations between 3 mM and 6 mM are required.

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# 5x Multiplex QPCR Mix (ROX)

## Attention!

**To activate the polymerase contained in the Multiplex QPCR Mix (ROX), you have to start your PCR with an incubation step for 15 minutes at 95 °C.**

Recommended standard temperature-time PCR profile

Step	Temperature	Time
Denaturation (D0)	95 °C	900 seconds
Denaturation (D1-35)	95 °C	30 seconds
Annealing/Extension (A/E1-35)	60 °C*	90 seconds
Number of cycles	35	

\* The best annealing / extension temperature depends on the detection system used.

## 5x Multiplex QPCR Mix (ROX)

### Attention!

**Note changes in concentration when multiplexing!**

The 5x multiplex QPCR mix is designed for a successful conventional multiplex PCR.

**For multiplexes with up to 13 primer pairs**, the following concentrations should be optimized:

- Increase the master mix concentration from 1x to 1.2x
- Titration of the F and R primer concentrations between 200 nM and 1 mM
- Titration of the Mg<sup>2+</sup> concentration from 3 mM to 6 mM

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## 5x Multiplex QPCR Mix (ROX)

The temperature-time profile differs depending on the melting temperature of the primer.

Temperature-time profile for primer mixes  $T_m > 60^\circ\text{C}$

Cycle step	Temperature	Time
Denaturation (D0)	95 °C	900 seconds
Denaturation (D1-35)	95 °C	30 seconds
Annealing (A1-35)	60 °C*	90 seconds
Extension (E1-35)	72 °C	30 seconds
Extension (Eend)	65 °C	30 -60 minutes*
Number of cycles		35

\* Necessary in order to equip all PCR fragments with an additional A.

## 5x Multiplex QPCR Mix (ROX)

Temperature-time profile for primer mixes  $T_m < 60^\circ\text{C}$

Cycle step	Temperature	Time
Denaturation (D0)	95 °C	900 seconds
Denaturation (D1-10)	95 °C	30 seconds
Annealing (TD1-10)	60 - 50 °C*	30 seconds
Extension (E1-10)	65 °C	60 seconds
Denaturation (D11-35)	95 °C	30 seconds
Annealing (A11-35)	50 °C*	30 seconds
Extension (E11-35)	65 °C	60 seconds
Extension (Eend)	65 °C	30-60 minutes*
Number of cycles	Touch Down (TD)-PCR 10 + 25 cycles	

\* Necessary to equip all PCR fragments with an additional A.

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# 5x Multiplex QPCR Mix (ROX)

## Technical Data

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**Shipment:** at room temperature

**Storage:** at -20 °Celsius

Storage at room temperature for up to 1 month does not affect the quality of the Hot-Start Taq DNA polymerase and the Bio&SELL 5x QPCR Mix (ROX)

**Precautionary Statement:** This product should only be used by people who are familiar with laboratory applications. Standard laboratory protective clothing such as gowns, gloves and protective goggles should be worn. In the event of contact with skin or eyes, the affected areas should be washed or rinsed out immediately with water.

**Application note:** In certain countries, some applications for which this product can be used are protected by patents. Since no licenses are acquired through the purchase, it may be necessary to acquire the corresponding license rights, depending on the country of use and the application.

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# 5x Multiplex QPCR Mix (ROX)

**Products for your research:**

## FBS SUPERIOR stabil®

Standardized Serum



**FBS SUPERIOR stabil® is our registered trademark at the Munich Patent and Trademark Office.**

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# 5x Multiplex QPCR Mix (ROX)

## Products for your research:

### Reverse Transcription incl QRT-PCR (One-Step Mixe)

#### **Bio&SELL**

#### **„One-Step RevTrans-qRT-PCR EvaGreen® No Rox“-Kit**

Combines reverse transcription with quantitative real-time PCR based on the EvaGreen® dye.

#### **Bio&SELL**

#### **„One-Step RevTrans-qRT-PCR EvaGreen® Rox“-Kit**

Combines reverse transcription with quantitative real-time PCR based on the EvaGreen® dye and also contains the internal reference dye Rox.

#### **Bio&SELL**

#### **„One-Step RevTrans-qRT-PCR Labeled Probes No Rox“-Kit**

Combines reverse transcription with probe-based quantitative real-time PCR.

#### **Bio&SELL**

#### **„One-Step RevTrans-qRT-PCR Labeled Probes Rox“-Kit**

Combines reverse transcription with probe-based quantitative real-time PCR and also contains the internal reference dye Rox.



**Save time and benefit  
of the low risk of  
contamination.**

[www.bio-sell.de/qrt-pcr/reverse-transkription-inkl-qrt-pcr-one-step-mixe.html](http://www.bio-sell.de/qrt-pcr/reverse-transkription-inkl-qrt-pcr-one-step-mixe.html)

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## Products for your research:

### RNA extraction products

#### **Bio&SELL**

##### **RNA Mini Kit**

For the isolation of highly pure RNA from tissue, cells and bacteria

#### **Bio&SELL**

##### **RNA Blood Kit**

For the isolation of highly pure RNA from whole blood or a leukocyte fraction

#### **Bio&SELL**

##### **RNA si/mi RNA Kit**

For the isolation of highly pure RNA from tissue, cells and bacteria.  
20 to 25 nucleotides 15 min preparation time

#### **Bio&SELL**

##### **RNA Tri-Liquid**

Classic, modified guanidine isothiocyanate / phenol method for isolating highly pure RNA from tissue, cells and bacteria

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