Protocol

Experimental recommendations for first use

Run a PCR with a temperature gradient at the annealing / extension step in order to find the optimal temperature for your assay respective each primer pair.

Table 1: Recommendations for PCR, qPCR and RT-PCR / Reaction Setup (20µL PCR reaction)

Component	Volume	Final concentration
LyoBall	0.75µL	1X (volume of 1 LyoBall)
Primer forward (10µM) *	1.0µL	0.5µM (0.05-1µM)
Primer reverse (10µM) *	1.0µL	0.5µM (0.05-1µM)
Probe (10µM) **	x µL	0.05-1µM
Template / sample extract ***	y µL	>0.1ng (0.1 – 2500 ng)
Nuclease-free water	up to 20µL total reaction volume	

Keep all components on ice.
Spin down and mix all solutions carefully before use.
** The necessary concentration of probes depends very much on the probe sequence and the kind of probe. Please test for optimum! Use temperature gradient to optimize the first time.
*** Recommended template concentration should be 0.001ng/µL – 0.1ng/µL (of genomic DNA).

Table 2: Typical qPCR protocol for amplification of DNA

Step	Temperature	Time
Initial denaturation	95°C	2 min.
2-step PCR Protocol in case of TM >60°C		
Denaturation	95°C	15 sec.
Annealing/Extension*	>60°C – 75°C	60 sec. (25 – 40 cycles)
Alternatively use a 3-step PCR Protocol in case of TM <60°C		
Denaturation	95°C	15 sec.
Annealing	55°C – 60°C	30 sec.
Extension*	68°C – 75°C	45 sec.(25 – 40 cycles)
Hold	<10°C	hold

NOTE: A two-step as well as a three-step PCR protocol can be used.

NOTE: Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used. It is highly recommended to establish a new RT-PCR by running a temperature gradient in order to find the best annealing/extension temperature for each new primer pair! Also a three-step PCR protocol can be applied with separate annealing and extension steps.

Important Notes

- LyoBalls may appear wet. This is due to a special developed ingredient, which is protecting the Balls from moisture uptake, however it does not interfere with the PCR reaction.
- LyoBalls dissolve within 1-2 minutes at room temperature without further/additional mixing.
- Master mixes, doesn't matter if liquid or lyophilized, from different companies contain different components which leads most likely to different annealing and melting behavior of DNA, primer and probes in your PCR! It will, most likely, not be possible to run the same PCR protocol with the LyoBalls as already established with another master mix.
- If you use our LyoBalls the first time or if you establish a new primer set, **please run a PCR with a temperature gradient** at the annealing / extension step in order to find the optimal temperature for your assay respective each primer pair.
- The annealing temperature of a primer is strongly influenced by its nucleic acid sequence AND the reaction composition (pH, salts and salt concentration of used reaction buffer). It has been shown that the annealing temperature is most likely lower by about 2-3°C using Genaxxon's LyoBalls compared to original systems!

The No ROX LyoBalls "master mix" is suited for example for following instruments:

Bio-Rad iCycler iQ, iQ5 and MyiQ™, CFX 96, CFX 480, Chromo 4™ Real-Time Detector, DNA Engine Opticon™, Mastercycler® ep realplex 2, Opticon® 2, Roche LightCycler® 96 and 480, Rotor-Gene™ / Corbet, QuantStudio™, Smart Cycler®