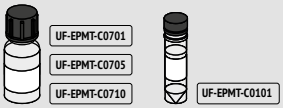
Takyon® Etheris Probe 2X qPCR Master Mix[1]

UF-EPMT-C0101	UF-EPMT-C0701	UF-EPMT-C0705	UF-EPMT-C0710
[1.5 mL] [150 x 20 µL RXNs]	[7.5 mL] [750 x 20 µL RXNs]	[5 packs of 7.5 mL]	[10 packs of 7.5 mL]

Kit content (for 750 (150[*]) - 20 µL reactions)

INCLUDED



Takyon® Etheris 2× Master Mix

- Black cap / Clear vial [7.5 mL]
- Black cap / Clear tube [1.5 ml][*]

- Takyon® HotStart DNA polymerase
- $MgCl_2$ (5.5 mM final concentration)
- dNTPs
- Stabilizers

OPTIONAL

ROX™

- Red cap / Amber tube [0.5 mL]

For signal normalization

NOT INCLUDED

Forward primer

Reverse primer

Water

Probe

Storage

Takyon® kit must be stored in a constant temperature freezer in the dark.

SHORT TERM STORAGE	LONG TERM STORAGE
6 Month stability	24 Month stability
4°C	-15°C -25°C

Optimization tips

Refer to the primer and probe design guidelines, custom assay design recommendations, primer titration matrix, and primer-probe ratio matrix for best results.

For new users of this kit, conducting a primer titration matrix may be required to ensure optimal performance.

↖ **TECHNICAL INFORMATION**

[1] Eurogentec products are sold for research or laboratory use only and are not to be administrated to humans or used for medical diagnostics.

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⚠ ROX™ Adjustment before first use

IMPORTANT

Before first use, convert your Master Mix to the correct ROX™ level.

Table 1 shows how much ROX™ passive reference to add to Takyon® Etheris qPCR Master Mix, according to the thermocycler used.

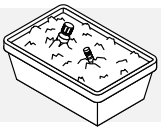
No ROX™ is needed for most thermocyclers not listed.

qPCR PLATFORM	1.5 ML MMX [ROX™ TO ADD]	7.5 ML MMX [ROX™ TO ADD]
Mx3000P®, 3005P, 4000	12 µL	60 µL
ABI Prism® 7500, FAST 7500, ViiA7™, QuantStudio™	4.2 µL	21 µL
ABI Prism® 5700, 7000,7300, 7700, 7900, FAST 7900, ABI Step One, One Plus MasterCycler® ep realplex I & II (rev. =< 2.1)	60 µL	300 µL

Table 1 : ROX™ addition according to qPCR Platform.

Recommended protocol

❶ Thaw all required reagents completely and put them **on ice.**

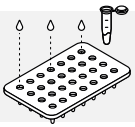


❷ Prepare the REACTION MIX in excess to correct for dispensing losses (*e.g.* a 100-reactions mix for 96-reactions). **Add all components together**, except for the template. Mix thoroughly by pipetting or inversion. **Spin down**.

	REACTION MIX COMPONENTS	VOLUME / REACTION	FINAL CONCENTRATION
	Takyon® MMx	5 µL	1×
	Forward primer	2 µL	50-900 nM[3]
	Reverse primer	2 µL	50-900 nM[3]
	Probe	2 µL	100-250 nM[3]
	RNAse-free Water	x µL[2]	(volume is 20 µL minus all other components)[4]
Total Mix/Reaction		**20 µL**	

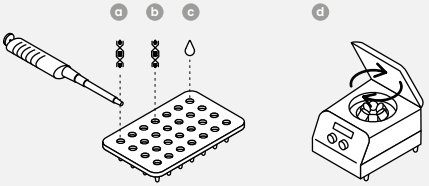
Table 2 : Reaction mix preparation.

❸ Add the **REACTION MIX** to individual reaction vials.



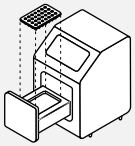
❹ Pipette into your qPCR plate/tubes either[4]:

ⓐ **2.5 µL of template cDNA / DNA** ⓑ **2.5 µL of positive control**
ⓒ **2.5 µL of water / buffer for negative control**



ⓓ Gently mix on a magnetic stirrer and centrifuge to avoid bubbles.

❺ Program the Real-Time thermocycler using the following recommended parameters:



Takyon® Etheris produces consistent and sensitive results under FAST[5] cycling conditions.

	T°C	TIME
Takyon® activation	95°C	3 min
40 CYCLES[5]		
Denaturation[6-7]	95°C	15 sec
Annealing / extension[7]	60°C	20 - 30 sec

Table 3 : Cycling conditions.

[2] Adjust water so total volume is 20 µL after adding all components. **[3]** Start with primers at 300 nM and probe at 250 nM; optimize if needed. **[4]** Mix 17.5 µL reaction mix with 2.5 µL DNA for a 20 µL final volume; adjust if required. **[5]** Use FAST cycling only on FAST-block cyclers; prefer amplicons <120 bp. **[6]** For complex templates (*e.g.,* plant/genomic DNA), increase denaturation time. **[7]** Set annealing temperature according to primer Tm. For longer/difficult templates, extend annealing/extension to 40 s. Some FAST cyclers allow shorter denaturation/annealing for quicker qPCR; if amplification is poor, lengthen extension in 5 s steps.