# Takyon® SYBR® MasterMix dTTP Blue with fluorescein[1]

| UF-FSMT-B0101 | UF-FSMT-B0701 | UF-FSMT-B0705 | UF-FSMT-B0710 |
|---|---|---|---|
| [1.5 mL] | [7.5 mL] | [5 x 7.5 mL] | [10 x 7.5 mL] |

## Kit content (for 750 (150) - 20 µL reactions)

INCLUDED

**2× Takyon® MasterMix**
● Green cap + ● Amber tube/vial
[7.5 mL]
[1.5 ml for UF-FSMT-B0101 ]

- Takyon® DNA polymerase
- $MgCl_2$ (2.5 mM final concentration)
- SYBR® Green
- dNTPs
- Inert blue dye
- Fluorescein
- Stabilizers

OPTIONAL

**1× 50 mM $MgCl_2$**
Clear cap
[ 1.5 mL ]

NOT INCLUDED

**Forward primer**

**Reverse primer**

**Water**

## Storage

SHORT TERM STORAGE

**6-month stability**
In the dark after kit production date

**4°C**

LONG TERM STORAGE

**12-month stability**
In the dark after kit production date

**-15°C | - 25°C**

qPCR reagents containing SYBR® Green should be protected from light during storage and qPCR assay setup.

## Optimization tips

Refer to the primer design guidelines, custom assay design recommendations, primer titration matrix, and $MgCl_2$ adjustment protocols for best results.
Upon developing a new assay or changing qPCR reagent kit, conducting a primer 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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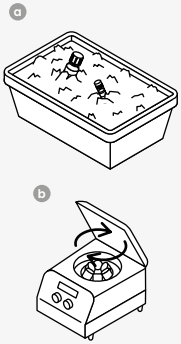
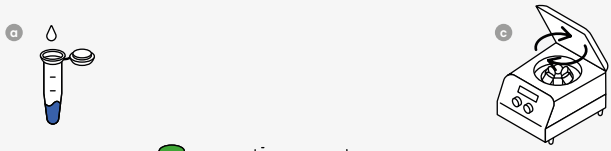
# Recommended protocol for 20-µL reactions

❶ ⓐ **Thaw all required reagents** completely and put them **on ice**. Mix all reagents well by inversion and ⓑ **spin down** the tube(s) prior to pipetting.

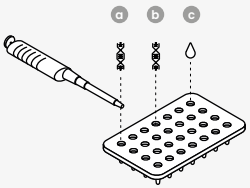



❷ ⓐ **Prepare the REACTION MIX** in excess to correct for dispensing losses (*e.g.* a 100-reactions mix for 96-reactions). ⓑ **Add all components together**, except for the template and controls. Mix gently by pipetting or inversion. ⓒ **Spin down.**




| REACTION MIX COMPONENTS | 2× Takyon® MasterMix | Forward primer | Reverse primer | Water | Total Volume |
|---|---|---|---|---|---|
| VOLUME / REACTION | 10 µL | 2 µL | 2 µL | 3.5 µL[2] | **17.5 µL[4]** |
| FINAL CONCENTRATION | 1× | 50-300 nM[3] | 50-300 nM[3] | (volume is 20 µL minus all other components)[2] | |

❸ **Pipette** into your qPCR plate/ vials either:

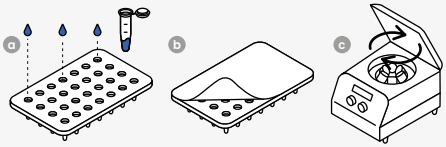



ⓐ **2.5 µL of template CDNA / DNA**

ⓑ **2.5 µL of positive control**

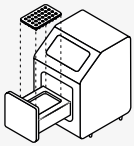
ⓒ **2.5 µL of water / buffer for negative control**

❹ ⓐ **Add 17.5 µL** of the **REACTION MIX** per well / vial.




ⓑ **Close the plate / vial** and mix gently on a stirrer and ⓒ **spin down**. Ensure that no bubbles are present in the reaction wells / vials.

❺ The Takyon® SYBR® MasterMix dTTP Blue with fluorescein will produce consistent and sensitive results under FAST and REGULAR cycling conditions. **Program the real-time thermocycler** using the following recommended parameters:



| | | FAST CYCLING[5] | REGULAR CYCLING |
|---|---|---|---|
| | T°C | Time | |
| Carry over prevention[6] (Optional) | 50°C | 2 min | 2 min |
| Takyon® activation | 95°C | 3 min | 3 min |
| **40 Cycles[7]** | | | |
| 2 STEPS Denaturation[8] | 95°C | 3 sec | 10 sec |
| 2 STEPS Annealing / extension[9] | 60°C | 20 - 30 sec | 45 - 60 sec |
| 3 STEPS Denaturation[8] | 95°C | 3 sec | 10 sec |
| 3 STEPS Annealing[9] | 60 °C | 15 sec | 20 sec |
| 3 STEPS Extension | 72 °C | 15 sec | 20 - 40 sec |

**[2]** Water volume is 20 µL minus volume of all other components. **[3]** Primers concentration of 100 nM is recommended as a starting concentration. This concentration will be correct for many assays, but additional optimization of the primers concentration may be required to obtain the best results with your primer set. **[4]** 17.5 µL of reaction mix is added to 2.5 µL of template/control DNA prior to cycling, giving a final reaction volume of 20 µL. See steps 3 and 4. These volumes, including primers and probes, can be adjusted depending on the template and reaction volumes. **[5]** Only perform fast cycling on FAST cyclers equipped with a FAST block. Short amplicons (<120 bp) are recommended to support FAST cycling conditions. For longer amplicons or difficult templates, increase the annealing-extension time up to 40 sec. **[6]** Applicable only if the dUTP/UNG Additive (Ref. RT-UTPUNG) has been added to the MasterMix. In such case, a temperature of at least 55°C should be maintained throughout the cycling protocol. **[7]** A 2-step protocol is recommended and effective in most cases. For challenging assays, optimize the primer matrix before considering a 3-step protocol. **[8]** Complex templates (plant DNA, genomic DNA...) may require a longer denaturation time. **[9]** The annealing temperature will vary depending on the melting temperature (Tm) of the primers. Note that some FAST thermocyclers can accommodate shorter annealing steps for faster qPCR results. However some assays may require longer extension times for efficient amplification. Increase extension time by increments of 5 second, if required.

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