TaqDog Hot Start 2X Green Master Mix

Products

Cat#	Description	Pack Size
TDHM001S	TaqDog Hot Start 2X Green Master Mix, 10 reactions, SAMPLE SIZE	0.25 mL
TDHM005	TaqDog Hot Start 2X Green Master Mix, 50 reactions of 50 µL size	1.25 mL
TDHM020	TaqDog Hot Start 2X Green Master Mix, 200 reactions of 50 µL size	5.0 mL
TDHM100	TaqDog Hot Start 2X Green Master Mix, 1,000 reactions of 50 µL size	25.0 mL

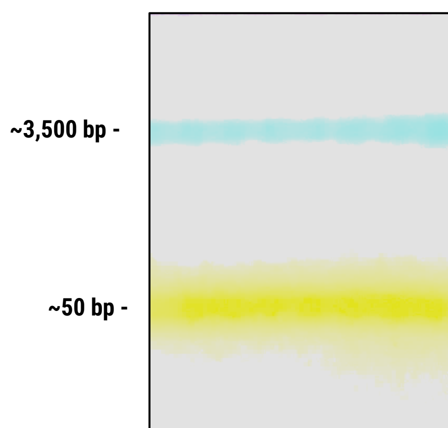
Product Information

The **TaqDog Hot Start 2X Green Master Mix** is a ready-to-use PCR mix with aptamer-based hotstart technology that minimizes the amplification of primer-dimers and nonspecific products. The aptamer contains a three-prime cap that prevents its amplification and ensures no interference with cloning or any downstream analysis. Please note that depending on the amount of master mix used, as well as staining sensitivity, the aptamers may be visible as a faint 50 base-pair band when imaged.

The **TaqDog Hot Start 2X Green Master Mix** is compatible with various DNA templates (including DNA of mouse or human origin, plasmid DNA, and bisulfite converted DNA) and leaves an adenine at the five-prime end – perfect for TA cloning.

Extension: 5,000 base-pair extension has been confirmed. *Speed:* Extension speeds up to 1 Kbp / minute.

TaqDog Hot Start 2X Green Master Mix contains a blend of two loading dyes (yellow and blue) that allows for loading and visualization during the run.



20 µl of diluted **TaqDog Hot Start 2X Green Master Mix** (1X) was loaded on a 1 % agarose gel and ran at 120 V for 30 minutes.

Product Components

	25.0 mL (1,000 reactions)	5.0 mL (200 reactions)	1.25 mL (50 reactions)	0.25 mL (10 reactions)
TaqDog 2 X Green Master Mix	20 x 1.25 mL	4 x 1.25 mL	1 x 1.25 mL	1 x 250 µL
TaqDog PCR Enhancer	4 x 1.0 mL	1 x 1.0 mL	1 x 1.0 mL	1 x 1.0 mL

Stored at - 20°C. Components may be freeze-thawed without significant loss of activity.

Protocol

Note: The following PCR protocol is a general outline suitable for many different templates, equipment, conditions, however optimal conditions for PCR should always be optimized for the specific template, equipment and conditions being used.

For new protocol development, always first optimize conditions by running at several annealing temperatures. The temperatures tested should range from the melting temperature of the primer all the way down to 7°C below the melting temperature. If primers have different melting temperatures use the **lower** of the two melting temperatures as reference.

1. Thaw the TaqDog Master Mix at room temperature. Vortex or invert to mix, then spin the tube briefly to collect the material at the bottom of the tube.
2. Add the following components to a PCR tube at room temperature:

Component	Volume	Concentration
Template DNA	Up to 10 µL	Up to 500 ng
Forward Primer (10 µM)	1 - 2.5 µL	200 – 500 nM
Reverse Primer (10 µM)	1 - 2.5 µL	200 – 500 nM
TaqDog Hot Start 2X Green Master Mix	25 µL	1x
TaqDog PCR Enhancer*	0 – 20 µL	-
Water, nuclease-free	up to 50 µL	-

**The PCR Enhancer is a specially formulated buffer that can be used to amplify difficult-to-amplify templates (%GC > 60) and/or remove non-specific amplification. The amount of PCR enhancer to use will vary depending upon the primer and template DNA being used and will need to be empirically determined. Please note annealing temperature requires re-optimization when using PCR enhancer.*

3. Mix contents (either through pipetting multiple times or brief vortexing).
4. Centrifuge briefly to bring all sample to bottom of tube.
5. When using a thermal cycler that does not use a heated lid (105 °C), overlay the reaction mixture with 25 µl of mineral oil.
6. Perform PCR using the following recommended guidelines:

Stage	Step	Temperature (°C)	Time	Cycles
1	Denature	94	30 sec	25 - 40
	Anneal	55-63	30 sec	
	Extend	72	1min/kB	
2	Final extension step	72	5 min	1
3	Holding step	4	∞	1

**Store at -20°C for longer term storage.*

6. PCR product is ready for downstream analysis.

END OF PROTOCOL

FAQ

Q1 Are all Master Mixes formulated for the same performance?
No. TaqDog 2x Green Master Mix contains no special additives so that performance for basic PCR using standard primer sets should be straightforward with few, if any changes to your existing protocols. However, every manufacturer's buffers/polymerase composition can behave differently, especially under more challenging PCR conditions. These conditions include: templates/primers with high GC content, dirty DNA preps, low levels of PCR inhibitors, etc. For these challenging conditions the optimal conditions for each master mix may diverge and the conditions for one may not work well for the other. If this is the case, an independent optimization should be made for TaqDog 2x Green Master Mix.

Q2 Should I inactivate the hot start element by an initial denaturation step?
No. TaqDog 2x Green Hot Start Master Mix uses an aptamer-based hot start technology. This element will efficiently disassociate as the first denaturation step is reached during the normal part of the thermal cycling program.

Q3 Do I need to make any changes to my PCR protocol if I need to use the Enhancer Reagent?
Likely yes. Commonly, optimization of the annealing temperature may be required when using the Enhancer Reagent. It is often sufficient to simply reduce the annealing temperature by 2 – 3°C. If still no band is visible after adjusting by 2 – 3°C, the optimal annealing temperature for the primers should be determined by running the PCR using annealing temperatures that span from the calculated melting temperature of the primers to 7°C below this temperature. If primers have different melting temperatures use the **lower** of the two melting temperatures as reference.

Q4 Do I need to optimize annealing temperature when using TaqDog 2x Green Master Mix?
In general, no. For new protocol development, always first optimize conditions by running at several annealing temperatures. The temperatures tested should range from the predicted melting temperature of the primer all the way down to 7°C below the melting temperature. If primers have different melting temperatures use the **lower** of the two melting temperatures as reference.

Q5 Do I need to optimize the number of cycles for PCR when using TaqDog 2x Green Master Mixes?
In general, no. You should always start at an expected/anticipated number of cycles. If this doesn't work, then you should increase the number of cycles by steps of 3. TaqDog 2x Green Master Mixes are effective between 25 and 40 cycles.

Q6 Why doesn't TaqDog 2x Green Master Mix work when I use my existing PCR protocol?
There are four likely reasons.
i) Your PCR conditions require the addition of the PCR Enhancer Reagent to help overcome inefficient amplification. You should empirically determine the optimal amount of PCR enhancer to use by titrating the amount added in increments of 5 μl, up to a maximum of 20 μl (In a 50 μl PCR reaction).
ii) In certain circumstances, optimization of the annealing temperature may be required. In the case of no visible amplification product, it is often sufficient to simply reduce the annealing temperature by 3°C. If still no band is visible after adjusting by 7°C, the optimal annealing temperature for the primers should be determined by running the PCR using annealing temperatures that span from the calculated melting temperature of the primers to 7°C below this temperature. If primers have different melting temperatures use the **lower** of the two melting temperatures as reference.
iii) If you were using a hot start master mix, such as Platinum Taq, then these types of hot start technologies require a long incubation at 95°C. On the other hand, the TaqDog Hot Start, as well as the TaqDog Premium 2x Green Master Mixes do not require any initial denaturation step. Simply set the denaturation temperature to 94°C for the number of cycles required to amplify the product.
iv) If no band is visible, please increase the number of cycles for the PCR protocol by increments of 3. TaqDog 2x Green Master Mixes are effective between 25 and 40 cycles.