STEMtaq™ Hot Start DNA Polymerase Master Mix Kit

Hot Start DNA polymerase master mix and nuclease-free water for PCR reactions at room temperature

Catalog #	79013	1 Kit	100 Reactions
	79013.1	1 Kit	1000 Reactions

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Product Description

STEMtaq™ Hot Start DNA Polymerase Master Mix Kit, which includes Hot Start master mix and nuclease-free water, reliably and consistently amplifies a wide range of PCR templates in a convenient master mix format that allows for room temperature setup. The enzyme is bound to a proprietary mouse-derived antibody that blocks activity until the initial denaturation step (94 - 95°C for 2 minutes) of the PCR re-activates the enzyme. Hot Start PCR can in some cases eliminate or minimize primer-dimers and secondary products for some targets, and potentially improve yield. STEMtaq™ Hot Start DNA Polymerase Master Mix is a 2X concentrated, ready-to-use solution containing the Hot Start version of STEMtaq™ DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer. STEMtaq™ Hot Start DNA polymerase has 5' to 3' exonuclease activity and produces PCR fragments with a 3' A overhang.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
STEMtaq™ Hot Start DNA Polymerase Master Mix Kit	79013	1 Kit - 100 Reactions	• STEMtaq™ Hot Start DNA Polymerase Master Mix (79014) • Nuclease-Free Water (79006)
STEMtaq™ Hot Start DNA Polymerase Master Mix Kit	79013.1	1 Kit - 1000 Reactions	• STEMtaq™ Hot Start DNA Polymerase Master Mix (79015) • Nuclease-Free Water (79012)

Component Storage and Stability

The following components are sold as part of a kit (Catalog #79013 or 79013.1) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMtaq™ Hot Start DNA Polymerase Master Mix*	79014	2 x 1.25 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
Nuclease-Free Water	79006	2 x 1.25 mL	Store at -20°C. Alternatively, store at < 30°C.	Stable until expiry date (EXP) on label.
STEMtaq™ Hot Start DNA Polymerase Master Mix*	79015	25 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
Nuclease-Free Water	79012	25 mL	Store at -20°C. Alternatively, store at < 30°C.	Stable until expiry date (EXP) on label.

*Contains DNA polymerase, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 4 mM $MgCl_2$, and reaction buffer (pH 8.5).

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Total DNA purification kit	e.g. Qiagen 69504
PCR tubes or plate	e.g. Corning PCR-02-C or PCR-96-C
Filtered pipette tips	e.g. 38035
Microcentrifuge tubes, 0.65 mL	e.g. 38037
Forward and reverse primers*	---
Thermocycler	---

*For assistance with primer design, visit www.stemcell.com.

Directions for Use

1. Purify DNA samples using a total DNA purification kit. Store on ice, or if not used immediately store at -20°C.
2. Thaw STEMtaq™ DNA Polymerase Master Mix and Nuclease-Free Water at room temperature (15 - 25°C).

   NOTE: If not used immediately, aliquot Master Mix and store at 2 - 8°C for up to 18 weeks, or at -20°C for long-term storage. Do not exceed expiry date as indicated on label. After thawing aliquots, use immediately. Do not re-freeze.
3. Vortex the Master Mix, then centrifuge briefly to collect material at the bottom of the tube.
4. The following example is for preparing 50 µL of Reagent Mix at room temperature. If preparing other volumes, adjust accordingly. Combine components in a microcentrifuge tube as indicated in Table 1.

Table 1. Reagent Mix Components

COMPONENT	VOLUME	FINAL CONCENTRATION
STEMtaq™ Hot Start DNA Polymerase Master Mix	25 µL	1X
Forward primer, 10 µM	0.5 - 5.0 µL	0.1 - 1.0 µM
Reverse primer, 10 µM	0.5 - 5.0 µL	0.1 - 1.0 µM
DNA template	1 - 5 µL	< 250 ng
Nuclease-Free Water	Variable	---
Total volume	**50 µL**	---

5. Centrifuge the Reagent Mix for 5 seconds.
6. Perform PCR in a thermocycler at room temperature. Refer to Table 2 for recommended PCR conditions. For PCR troubleshooting, see Notes and Tips.

   NOTE: Initial denaturation step (95°C for 2 minutes) is required for activation of the Hot Start polymerase.

Table 2. Recommended PCR Cycling Conditions

STEP	TEMPERATURE	TIME
Initial denaturation	95°C	2 minutes
Denaturation, annealing, extension for 25 - 30 cycles	95°C	15 - 60 seconds
	Varies ~5°C below the lowest melting temperature of the primers (Tm)	15 - 60 seconds
	72 - 74°C	1 minute per kb to be amplified
Final extension	72 - 74°C	5 minutes
Hold	4°C	Infinite

7. Store the reaction products at 2 - 8°C for up to 24 hours or at -20°C for long-term storage.

Notes and Tips

PCR Troubleshooting

If PCR results in low amplification/no amplification of DNA, try any of the following:

- Decrease annealing temperature/increase annealing time
- Increase number of PCR cycles
- Increase concentration of primer, template, and/or polymerase
- Minimize the effect of inhibitors by diluting DNA template or using less. Alternatively, use an ethanol precipitation and wash step on DNA template prior to PCR.

- Add PCR-enhancing agents (e.g. DMSO or betaine) or a stabilizing agent such as BSA (to a final concentration of 0.16 mg/mL)

If non-specific bands are obtained:

- Increase annealing temperature
- Increase primer length to increase specificity
- Adjust annealing time:
  - If non-specific bands are longer than target, decrease annealing time
  - If non-specific bands are shorter than target, increase annealing time

Related Products

For related products, including genome editing tools, qPCR arrays, specialized cell culture and storage media, and cultureware, visit www.stemcell.com or contact us at techsupport@stemcell.com.