



## PCR reagent starter pack

Cat. No.: 42-521

### Congratulations on your new PCR cycler!

To help you get started quickly and confidently, this PCR reagent starter pack provides a curated selection of high-performance enzymes and reagents for reliable and reproducible results.

The kit includes a range of ready-to-use master mixes covering standard PCR, hot-start applications, and high-fidelity amplification, as well as a convenient clean-up solution for downstream workflows. Together, these products support optimal performance across a wide variety of PCR applications.

| Item | Product name                               | Format / Features                                | Reactions |
|------|--------------------------------------------|--------------------------------------------------|-----------|
| 1    | 2.0X Taq RED Master Mix Kit                | Includes loading dye (1.5 mM MgCl <sub>2</sub> ) | 20        |
| 2    | 2.0X Taq Master Mix, Clear                 | Standard PCR mix (1.5 mM MgCl <sub>2</sub> )     | 20        |
| 3    | Apex Hot Start High Fidelity 2X Master Mix | High accuracy amplification                      | 20        |
| 4    | Apex Hot Start 2.0X Master Mix Blue        | Hot start, includes loading dye                  | 20        |
| 5    | Apex One-Step PCR Clean-Up Kit             | Post-PCR clean-up                                | 10        |



## 2.0X Taq RED Master Mix Kit (1.5mM MgCl<sub>2</sub>) Cat #: SMP42-138

**Contents:** 20 Reactions

**Storage:** -20°C.

Reagent for *in vitro* laboratory use only

### General Description

Apex Taq RED DNA Polymerase Master Mix from Genesee Scientific is a ready-to-use 2.0X reaction mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications. Apex Taq DNA Polymerase, the NH<sub>4</sub><sup>+</sup> buffer system, dNTPs and magnesium chloride are present in the Taq RED DNA Polymerase Master Mix. Each reaction requires 25 µl of the 2.0X reaction mix. Simply add primers, template and water to a total reaction volume of 50 µl.

Taq RED DNA Polymerase Master Mix offers several advantages. Set up time is significantly reduced. There is no need to buy and use separate loading dyes to load reaction products onto agarose gels for electrophoresis and subsequent visualization. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

### Composition of the 2.0X Taq RED Master Mix (1.5 mM MgCl<sub>2</sub>)

- Tris-HCl pH 8.5, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3 mM MgCl<sub>2</sub>, 0.2 % Tween® 20
- 0.4 mM of each dNTP
- Apex Taq DNA polymerase
- Inert red dye and stabilizer

### Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

Option: Store at +4 °C for up to 6 months.

### Quality Control

Taq DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

### Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

### Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- The table below shows the reaction set up for a final volume of 50 µL.
- **Important:** Mix the solutions completely before use to avoid localized concentrations of salts.

1. Set up each reaction as follows:

| Component               | Vol./Reaction | Final Conc. |
|-------------------------|---------------|-------------|
| 2.0X Taq RED Master Mix | 25 µL         | 1X          |
| Primer A                | Variable      | 0.1–1.0 µM  |
| Primer B                | Variable      | 0.1–1.0 µM  |
| Nuclease-Free Water     | Variable      | ----        |
| Template DNA            | Variable      | Variable    |
| <b>TOTAL volume</b>     | 50 µL         | ----        |

2. Mix gently by pipetting the solution up and down a few times.
3. Program the thermal cycler according to the manufacturer's instructions.

For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

**Table 3. Three-step PCR program**

| Cycles | Duration of cycle                                                                     | Temperature                  |
|--------|---------------------------------------------------------------------------------------|------------------------------|
| 1      | 2-5 minutes                                                                           | 95 °C                        |
| 25-35  | 20 – 30 seconds <sup>a</sup><br>20-40 seconds <sup>b</sup><br>30 seconds <sup>c</sup> | 95 °C<br>55 – 60 °C<br>72 °C |
| 1      | 5 minutes <sup>d</sup>                                                                | 72 °C                        |

<sup>a</sup>. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

<sup>b</sup>. Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the T<sub>m</sub> (melting temperature) of the primers used.

<sup>c</sup>. Extension/elongation step: Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

<sup>d</sup>. Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.



## 2.0X Taq Master Mix, Clear (1.5mM MgCl<sub>2</sub>) Cat #: SMP42-134

**Contents:** 20 Reactions

**Storage:** -20°C.

Reagent for *in vitro* laboratory use only

### General Description

Apex Taq DNA Polymerase Master Mix, Clear from Genesee Scientific is a ready-to-use 2.0X reaction mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications.

Apex Taq DNA Polymerase, an optimized NH<sub>4</sub><sup>+</sup> buffer system, dNTPs and magnesium chloride are present in the Taq DNA Polymerase Master Mix, Clear. Each reaction requires 25 µl of the 2.0X reaction mix. Simply add primers, template and water to a total reaction volume of 50 µl.

Taq DNA Polymerase Master Mix, Clear offers several advantages. Optimized for increased specificity of DNA targets up to 3 kb. Setup time is significantly reduced. The chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

### Composition of the Taq Master Mix, Clear (2.0X)

- Tris-HCl pH 8.5, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3 mM MgCl<sub>2</sub>, 0.2 % Tween® 20
- 0.4 mM of each dNTP
- Apex Taq DNA polymerase

### Storage and Stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

Option: Store at +4 °C for up to 6 months.

### Quality Control

Taq DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity or exonuclease activity.

### Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

### Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- The table below shows the reaction set up for a final volume of 50 µL.
- Keep all components on ice.
- **Important:** Mix the solutions completely before use to avoid localized concentrations of salts.

1. Set up each reaction as follows:

| Component                  | Vol./Reaction | Final Conc. |
|----------------------------|---------------|-------------|
| 2.0X Taq Master Mix, Clear | 25 µL         | 1X          |
| Primer A                   | Variable      | 0.1–1.0 µM  |
| Primer B                   | Variable      | 0.1–1.0 µM  |
| Nuclease-Free Water        | Variable      | ----        |
| Template DNA               | Variable      | Variable    |
| <b>TOTAL volume</b>        | 50 µL         | ----        |

2. Mix gently by pipetting the solution up and down a few times.
4. Program the thermal cycler according to the manufacturer's instructions.

For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

**Table 3. Three-step PCR program**

| Cycles | Duration of cycle                                                                     | Temperature                  |
|--------|---------------------------------------------------------------------------------------|------------------------------|
| 1      | 2-5 minutes                                                                           | 95 °C                        |
| 25-35  | 20 – 30 seconds <sup>a</sup><br>20-40 seconds <sup>b</sup><br>30 seconds <sup>c</sup> | 95 °C<br>55 – 60 °C<br>72 °C |
| 1      | 5 minutes <sup>d</sup>                                                                | 72 °C                        |

<sup>e</sup>. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 95 °C for 20 – 30 seconds. It causes melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

<sup>f</sup>. Annealing step: The reaction temperature is lowered to 50 – 65 °C for 20 – 40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically, the annealing temperature is about 3 – 5 °C below the T<sub>m</sub> (melting temperature) of the primers used.

<sup>g</sup>. Extension/elongation step: Taq polymerase has its optimum activity temperature at 72 °C. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand. The extension time depends on the length of the DNA fragment to be amplified. As a rule of thumb, at its optimum temperature the DNA polymerase will polymerize a thousand bases per minute.

<sup>h</sup>. Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

## Part 3 of 5



# Apex Hot Start High Fidelity 2X Master Mix Cat #: SMP42-513

**Contents:** 20 Reactions

**Storage:** -20°C.

Reagent for *in vitro* laboratory use only

## Contents

All-in-one 2x master mix containing the Apex Hot Start High Fidelity DNA Polymerase, optimized Buffer, dNTPs and MgCl<sub>2</sub>. Recommended for low-bias, high fidelity amplification.

## Recommended Storage and Stability

- Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.
- Store at room temperature for up to 5 days.
- Store at +4 °C for up to 6 months.

## Protocol

- Apex Hot Start High Fidelity 2X Master Mix is not frozen at -20 °C. Simply mix the tube thoroughly and it is ready for preparation of the reaction mix.
- **Very important:** It is critical not to use too high a concentration of primer to reduce unspecific amplification. With this product you might need to reduce the primer concentration compared to other similar master mixes that you might have been using previously.

1. Combine master mix, primers, template DNA and water according to the following table.

**Table 1:** Recommended reaction setup

| Component                  | Vol./reaction*  | Final concentration*                                                                                    |
|----------------------------|-----------------|---------------------------------------------------------------------------------------------------------|
| 2x Master Mix              | 25 µl           | 1x                                                                                                      |
| Primer A (10 µM)           | 1 µl            | 0.2 µM                                                                                                  |
| Primer B (10 µM)           | 1 µl            | 0.2 µM                                                                                                  |
| 25 mM MgCl <sub>2</sub>    | 0 µl (0 – 5 µl) | 2.0 mM (2.0 – 4.5 mM)                                                                                   |
| Betaine (5 M)**            | 0 – 5 µl        | 0 – 0.5 M                                                                                               |
| PCR-grade H <sub>2</sub> O | X µl            | -                                                                                                       |
| Template DNA               | X µl            | genomic DNA: 50 ng (10 – 500 ng)<br>plasmid DNA: 0.5 ng (0.1 – 1 ng)<br>bacterial DNA: 5 ng (1 – 10 ng) |
| <b>TOTAL volume</b>        | 50 µl           | -                                                                                                       |

\* Suggested starting conditions; theoretically used conditions in brackets.

\*\* Suggested for GC-rich amplification. See section *Strategies for Optimization*.

2. Transfer the appropriate volume to a PCR plate or strip compatible with the chosen thermal cycler. Seal the plate/strip.

## 3. Three-step PCR program

**Table 2:** Recommended cycling conditions

| Step                 | Duration of cycle                                                                   | Temperature                  |
|----------------------|-------------------------------------------------------------------------------------|------------------------------|
| Initial denaturation | 30 sec – 2 min                                                                      | 98 °C                        |
| 25 – 35 cycles       | 10 – 20 sec <sup>a)</sup><br>15 – 30 sec <sup>b)</sup><br>10 – 60 sec <sup>c)</sup> | 98 °C<br>55 – 70 °C<br>72 °C |
| Final elongation     | 5 min                                                                               | 72 °C                        |

<sup>a)</sup> Denaturation: During thermocycling, 10 seconds usually works very well. Longer denaturation times might be required for long range PCR or amplification from templates with a high GC content.

<sup>b)</sup> Primer annealing: Typically, the annealing temperature is about 3 – 5 °C below the T<sub>m</sub> (melting temperature) of the primers used. **Because of the high salt content within the Apex Hot Start High Fidelity 2X Master Mix, annealing temperature will likely be higher than with more traditional PCR buffers.**

<sup>c)</sup> Extension: The recommended extension temperature is 72°C. Extension times highly depends on the length of the amplicon. **Generally, we recommend an extension time of 10-30 seconds per kb for complex genomic targets. 10 seconds per kb is often sufficient for simpler targets (such as plasmids) or short complex targets (< 3 kb). 30-60 seconds per kb is recommended for long amplicons (> 3 kb).**

## 4. Two-step PCR program

For targets with annealing temperatures ≥ 72°C (usually GC-rich primers), a 2-step thermocycling protocol (combining annealing and extension into one step) can be used.

## Strategies for Optimization

### High-yield/Nonspecific amplification

- Decreased primer concentration can improve PCR product specificity of long amplicons.
- Decreased extension time can improve PCR product specificity of short amplicons.
- Decreased PCR cycles will decrease PCR product yield.

### Long-range amplification

- Longer extension times often resolve low-yield amplification of long amplicons.
- Increased template concentration will increase product yield.
- Increased number of PCR cycles will increase product yield.
- Increased primer concentration can increase product yield for some reactions.

### GC-rich amplification

- The addition of 0.5 M Betaine solution often improves reaction performance.
- Primers of 20 – 40 nucleotides with a GC content of 40 – 60 % are recommended. Online Software such as the [Primer3plus](#) can be used to design primers.

### MgCl<sub>2</sub>

The optimal MgCl<sub>2</sub> concentration should be determined empirically but in most cases a concentration of 2.0 mM, as provided in this Master Mix (1x), will produce satisfactory results.



## Apex Hot Start 2.0X Master Mix Blue

with Apex Buffer I

**Cat #: SMP42-144**

**2.0X Master Mix Kit (1.5mM Final MgCl<sub>2</sub> Conc.)**

**Contents:** 20

**Storage:** -20°C.

Reagent for *in vitro* laboratory use only

### General Description

Apex Hot Start Master Mix BLUE is a ready-to-use 2.0X master mix. Simply add primers, template and water to successfully carry out primer extensions and other molecular biology applications.

Apex Hot Start DNA Polymerase, NH<sub>4</sub><sup>+</sup> buffer system, dNTPs and magnesium chloride are present in Apex Hot Start Master Mix with Apex Buffer I. Each reaction requires 25 µL of the 2.0X reaction mix. Simply add primers, template and water to a total reaction volume of 50 µL.

Apex Hot Start DNA Polymerase is a modified form of Apex Taq DNA Polymerase, which is activated by heat treatment. A chemical moiety is attached to the enzyme at the active site, which renders the enzyme inactive at room temperature. Thus, during setup and the first ramp of thermal cycling, the enzyme is not active and misprimed primers are not extended. The result is higher specificity and greater yields when compared to standard DNA polymerases.

Apex Hot Start Master Mix BLUE offers several advantages: Direct gel loading, no need to use separate loading dyes for electrophoresis and subsequent visualization, and the chance of contaminating component stocks is eliminated. Reduction of reagent handling steps leads to better reproducibility. Standard tests can be set up with the confidence that results will be consistent every time.

### Composition of 2.0 X Apex Hot Start Master Mix BLUE w/ buffer I

- Tris-HCl, pH 8.5, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3 mM MgCl<sub>2</sub>, 0.2% Tween 20®.

- 0.4 mM dNTPs
- Apex Hot Start DNA Polymerase
- Inert Blue Dye
- Stabilizer

### Protocol

This protocol serves as a guideline for primer extensions. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be individually determined.

### Notes:

- Set up reaction mixtures in an area separate from that used for DNA preparation or product analysis.
- The table below shows the reaction set up for a final volume of 50 µL.
- **Important:** Mix the solutions completely before use to avoid localized concentrations of salts.

1. Set up each reaction as follows:

| Component                                         | Vol./Reaction | Final Conc. |
|---------------------------------------------------|---------------|-------------|
| Apex Hot Start Master Mix Blue with Apex Buffer I | 25 µL         | 1X          |
| Primer A                                          | Variable      | 0.1–1.0 µM  |
| Primer B                                          | Variable      | 0.1–1.0 µM  |
| PCR Grade Water                                   | Variable      | ---         |
| Template DNA                                      | Variable      | Variable    |
| <b>TOTAL volume</b>                               | 50 µL         | ---         |

2. Mix gently by pipetting the solution up and down a few times.
3. Program the thermal cycler according to the manufacturer's instructions.
4. **Each program must start with an initial heat activation step at 95°C for 15 minutes.**

For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.

A typical thermal cycling program is shown below:

|                         |          |                                    |
|-------------------------|----------|------------------------------------|
| <b>95°C for 15 min.</b> |          | Activate Apex Hot Start Polymerase |
| <b>30-40 cycles:</b>    |          |                                    |
| <b>95°C</b>             | 30 sec.  | Denature template                  |
| <b>45-65°C</b>          | 30 sec.  | Anneal primer                      |
| <b>72°C</b>             | 1-5 min. | Elongation                         |
| <b>72°C for 5 min.</b>  |          | Elongation                         |

5. Place the tubes in the thermal cycler and start the reaction.



## Apex One-Step PCR Clean-Up Kit

### Cat #: SMP42-505

**Contents:** 1 x 0.02 ml, 10 reactions

**Storage:** -20°C.

Reagent for *in vitro* laboratory use only

#### General Description

Apex One-Step PCR Clean-Up Kit consists of a heat labile Exonuclease I (HL-ExoI) and a recombinant Shrimp Alkaline Phosphatase (rSAP). Treatment of PCR products with Apex One-Step PCR Clean-Up Kit, removes residual primers and dNTPs.

Add Apex One-Step PCR Clean-Up Kit directly to the reaction containing the amplified PCR product. After treatment at 37 °C for minimum 2 minutes, Apex One-Step PCR Clean-Up Kit is completely inactivated by heating at 80 °C for minimum 3 minutes. No sample loss is observed after treatment with Apex One-Step PCR Clean-Up Kit.

#### Composition of Apex One-Step PCR Clean-Up Kit

- Balanced mixture of a heat labile Exonuclease I (HL-ExoI) and a recombinant Shrimp Alkaline Phosphatase (rSAP).

#### Storage and Stability

Store at -20 °C. Product expiry at -20 °C is stated on the label.

#### Quality control

Apex One-Step PCR Clean-Up Kit is functionally tested by spiking a PCR product with dNTPs and primers followed by sanger sequencing by capillary electrophoresis. HL-ExoI and rSAP are tested individually for double stranded and single stranded endonuclease activity.

#### Protocol

This protocol serves as a guideline for clean-up of 5 µl PCR product using Apex One-Step PCR Clean-Up Kit\*.

1. Take out Apex One-Step PCR Clean-Up Kit from the -20 °C freezer.
2. Keep Apex One-Step PCR Clean-Up Kit on ice at all times.
3. Add 2 µl Apex One-Step PCR Clean-Up Kit\*\* to 5 µl of amplified PCR product.
4. Mix well and spin down.
5. Incubate the reaction at 37 °C for 2 - 5 minutes to degrade remaining primers and to inactivate excess nucleotides by dephosphorylation.
6. Incubate at 80 °C for 3 - 10 minutes to completely inactivate Apex One-Step PCR Clean-Up Kit.
7. The cleaned up PCR product can now be used for downstream applications such DNA sequencing, primer extension experiments or SNP analysis.
8. After treatment the PCR products can be stored at -20 °C.

\*If treating PCR product of higher volume, then increase proportionally the amount of Apex One-Step PCR Clean-Up Kit. \*\*Apex One-Step PCR Clean-Up Kit works in PCR buffers.