



Catalog #	Aliquot Size
HF01-E012-1	1 mL
HF01-E012-5	5 x 1 mL
HF01-E012-15	15 x 1 mL

## High Fidelity Master Mix (Dye Plus)

Catalog # HF01-E012

Lot # A4647-4

### Product Description

High Fidelity Master Mix (Dye Plus) is a special next generation premix for PCR. This product contains 2X master mix to which primers and template can be added. It produces rapid target amplification (4 - 5 sec/kb) while maintaining high fidelity and yield. A loading dye in the mix facilitates easy loading and tracking of DNA samples in agarose or native polyacrylamide gels.

### Formulation

This master mix contains DNA Polymerase, dNTPs, an optimized buffer system with two types of monoclonal antibodies, and a loading dye in a proprietary formulation.

### Storage and Stability

Store at -30°C to -15°C. To avoid repeated handling and multiple freeze/thaw cycles aliquot product into smaller quantities.

### Scientific Background

Compared to Taq DNA Polymerase, High Fidelity DNA polymerase has better specificity, higher yield, and faster amplification performance (4 - 5 sec/kb). Its amplification error rate is 81-fold lower than that of Taq. Matched with an optimized buffer system, this master mix can be used for PCR amplification of templates such as genomic DNA, cDNA, plasmid DNA, dU-containing DNA, and crude samples. It has excellent compatibility with GC-rich primer/template systems. This master mix contains two types of monoclonal antibodies, inhibiting the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, which enable it to perform hot start PCR with great specificity. Amplification will generate blunt-ended products. A loading dye in the master mix allows the PCR products to directly be loaded for electrophoresis after the reaction. On a typical 1% agarose gel in a 1X TAE buffer or TBE buffer, the dye front migrates at the same rate as a DNA fragment of about 400 base pairs (Blue band ~400bp).

**This product is manufactured in an ISO 9001 and ISO 13485 certified facility.**

## High Fidelity Master Mix (Dye Plus)

Catalog #	HF01-E012
Lot #	A4647-4
Stability	1yr from date of shipment at -30°C to -15°C.
Storage & Shipping	Store at -30°C to -15°C. Transport at ≤0 °C. To avoid repeated handling and multiple freeze/thaw cycles aliquot product into smaller quantities.

To place your order, please contact us by phone 1-778-326-0223 or 1-888-606-3424 (Toll free) or by email: [orders@signalchemdx.com](mailto:orders@signalchemdx.com) or [info@signalchemdx.com](mailto:info@signalchemdx.com) - [www.signalchemdx.com](http://www.signalchemdx.com)

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# PCR Protocol

## Guidelines for primer design:

1. It is recommended that the last base at the 3' end of primer should be G or C.
2. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
3. Avoid hairpin structures at the 3' end of the primer.
4. Differences in the T<sub>m</sub> value of the forward primer and the reverse primer should be no more than 1°C and the T<sub>m</sub> value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the T<sub>m</sub> value).
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer T<sub>m</sub> value.
6. Control the GC content of the primer to be 40% - 60%.
7. The overall distribution of A, G, C and T in the primer should be as even as possible. Avoid using regions with high GC or AT contents.
8. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers and avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
9. Use the NCBI BLAST function to check the specificity of the primer to prevent non-specific amplification.

## Protocol:

### 1. General reaction mixture for PCR:

Keep all components on ice. Thaw, mix and briefly centrifuge each component before use.

Component	Volume in 50 µl system
2X High Fidelity Master Mix (Dye Plus)	25 µl
Primer 1 (10 µM)	2 µl
Primer 2 (10 µM)	2 µl
Template DNA*	Variable
ddH <sub>2</sub> O	to 50 µl

\* Optimal reaction concentration varies for different templates. The following table shows the recommended template concentration for a 50µl reaction system:

Template Type	Amount
Genomic DNA	10-500 ng
Plasmid or Virus DNA	5 pg-20 ng
cDNA	1 - 5 µl (≤1/10 of the total volume of PCR system)

Note: High quality templates should be used to ensure successful amplification and product yield.

The DNA Polymerase in this master mix has strong proof-reading activity. If TA cloning needs to be performed, please perform purification before adding an A-tail.

### 2. PCR Thermocycling conditions:

#### Standard Program

Steps	Temperature	Time	Cycle number
Initial Denaturation	98°C	30 sec	
Denaturation	98°C	10 sec	} 28-35 cycles
Annealing <sup>a</sup>	T <sub>m</sub>	5 sec	
Extension <sup>b</sup>	72°C	4-5 sec / kb	
Final Extension	72°C	1 min	

#### Fast Program <sup>c</sup>

Steps	Temperature	Time	Cycle number
Denaturation	98°C	10 sec	} 28-35 cycles
Annealing <sup>a</sup>	T <sub>m</sub>	5 sec	
Extension <sup>b</sup>	72°C	4-5 sec / kb	

- a. Set the annealing temperature according to the T<sub>m</sub> value of the primers. If the T<sub>m</sub> value of the primers is higher than 72°C, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized through setting a temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve amplification specificity.

- b. Set the extension time according to the following table:

Target fragment size	Extension time
≤10 kb	4 - 5 sec/kb
>10 kb	10 sec/kb

- c. Through experimental verification, there is no significant difference in performance when adopting either standard program or fast program. You can choose according to your operating habits.

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## FAQ & Troubleshooting:

	<b>No amplification products or low yield</b>	<b>Unspecific products or smear bands</b>
Primer	Optimize primer design	Optimize primer design
Annealing temperature	Set annealing temperature gradient and find the optimal annealing temperature	Increase the annealing temperature and set gradient annealing temperature
Primer concentration	Increase the primer concentration appropriately	Decrease the primer concentration appropriately
Extension time	Increase the extension time up to 10 - 15 sec/kb appropriately	
Cycles	Increase the number of cycles up to 36 - 40 cycles	Reduce the number of cycles to 25 - 30 cycles
PCR Program		Use two-step or Touch down PCR program
Template purity	Use templates with high purity	Use templates with high purity
Input amounts of template	Adjust the template amount according to the recommend amount and appropriately increase the amount	Adjust the template amount according to the recommend amount and appropriately decrease the amount

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# SAFETY DATA SHEET

## Article 1 - Product Identification

**Product Name: High Fidelity Master Mix (Dye Plus)****Catalog # HF01-E012**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Supplier of Datasheet: SignalChem Diagnostics Inc.  
Street Address: 190-13160 Vanier Place  
City, Prov. Postal Code: Richmond, BC, V6V 2J2  
Country: Canada  
Emergency Phone: 1-888-606-3424 (Toll free)  
1-778-326-0223 (local)

## Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Not GHS classified.
- **Hazard Pictograms:** No labeling applicable.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** Wear protective gloves/protective clothing/eye protection/ face protection. Avoid breathing dust. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- **Other hazards:** May cause eye and skin irritation. May cause respiratory and digestive tract irritation.

## Article 3 – Composition/Information on Ingredients

**Chemical Characterization:** Mixture.

**Description:** The components of this product which may be hazardous are listed below.

Common name	Chemical name	CAS-No.	Concentration
Water	H <sub>2</sub> O	7732-18-5	≤95%
Potassium Chloride	KCl	7447-40-7	≤2%
Glycerol	Glycerol	56-81-5	≤1%
Tris	TRIS(Hydroxymethyl)Aminomethane	77-86-1	≤1%
DMSO	Dimethyl sulfoxide	67-68-5	≤0.8%
Magnesium Chloride	MgCl <sub>2</sub>	7791-18-6	≤0.1%

## Article 4 – First-aid Measures

- **General information:** Consult a physician and provide this SDS.
- **After inhalation:** In case of irritation by inhaling this product, move affected person to fresh air and await recovery. If irritation persists, seek immediate medical attention. If casualty cannot breathe, give artificial respiration and seek immediate medical attention.
- **After skin contact:** Wash with clean water, immediately.
- **After eye contact:** Flush eyes with clean water for more than 15 minutes and seek medical attention.
- **After swallowing:** Induce vomiting. If indisposition continues, seek medical attention.

## Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Water, Carbon Dioxide, Foam, Dry Chemical Powder
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

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## Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains. Do not discharge directly into sewers.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

## Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store according to product label instructions. Keep container upright and tightly closed.

## Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**  
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m <sup>3</sup> for mist	British Columbia, Canada
TWA	3 mg/m <sup>3</sup> for respirable mist	British Columbia, Canada
TWA	10 mg/m <sup>3</sup>	Alberta, Canada
TWAEV	10 mg/m <sup>3</sup>	Ontario, Canada
TWAEV	10 mg/m <sup>3</sup>	Quebec, Canada
TWA	10 mg/m <sup>3</sup>	USA

- **Appropriate engineering controls:**  
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**  
**Respiratory protection:**  
No special protective equipment required. If ventilation is poor, wear respirators that are suitable according to government standards.  
**Hand protection:**  
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.  
**Eye/face protection:**  
Safety goggles with side-shields approved under appropriate government standards.  
**Skin/body protection:**  
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

## Article 9 – Physical and Chemical Properties

<b>Appearance:</b> Colorless liquid.	<b>Danger of explosion:</b> Product does not present an explosion hazard.
<b>Odour/Odour Threshold:</b> Not determined.	<b>Explosion limits:</b> Not available.
<b>pH:</b> Not available.	<b>Decomposition temperature:</b> Not available.
<b>Melting point/freezing point:</b> Not determined.	<b>Vapor pressure at 20 °C:</b> Not available.
<b>Boiling point/Boiling range:</b> Not determined.	<b>Density:</b> Not determined.
<b>Flash point:</b> Not determined.	<b>Relative density:</b> Not determined.
<b>Flammability (solid, gaseous):</b> Not determined.	<b>Vapor density:</b> Not determined.
<b>Ignition temperature:</b> Not determined.	<b>Evaporation rate:</b> Not determined.
<b>Auto-igniting:</b> Product is not self-igniting.	<b>Solubility in / Miscibility with Water:</b> Fully miscible.

## Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing agents.
- **Hazardous decomposition products:** Not determined.

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# SAFETY DATA SHEET

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## Article 11 - Toxicological Information

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- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  - Inhalation: No data available
  - Ingestion: No data available
  - Skin: No data available
  - Eyes: No data available
- **Signs and Symptoms of Exposure:** No data available
- **Synergistic effects:** Not available.

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## Article 12 - Ecological Information

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- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

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## Article 13 - Disposal Considerations

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- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

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## Article 14 - Transport Information

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- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

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## Article 15 - Regulatory Information

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- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

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## Article 16 - Other Information

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The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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