USER GUIDE

5X PCR Master Mix

Cat. ADPMX01-100
Qty. 500 µl

Content and Storage

Content	Cat	Product	Qty
	ADPMX01-100	5X PCR Master Mix	500 µl

Storage stored in a stable **-20** ℃

Composition of 5X PCR Master Mix

150 mM Tris-HCl pH 8.5,
40 mM (NH4)2S04,
1.5 mM MgCl2,
0.2% Tween 20
0.4 mM dNTPs0
0.05 units/µ *Taq* DNA polymerase

Description

5X PCR Master Mix is a ready-to-use 5.0x reaction mix. Simply add primers, template, and water to successfully carry out primer extensions and other molecular biology applications. Engine *Taq* polymerase, the NH4+ buffer system, dNTPs and magnesium chloride are conveniently present in the Taq DNA Polymerase Master Mix . Taq DNA Polymerase Master Mix , offers several advantages. Set up 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.

ARROWTEC

Standard Protocol

1. Set up each reaction as follows:

component	Vol./reaction	Final Conc.
5X PCR Master Mix	5 µl	1X
Primer A	Variable	0.1-1.0 µM
Primer B	Variable	0.1-1.0 µM
Distilled Water	Variable	---------
Template DNA	Variable	Variable
TOTAL Volume	25 µl	---------

2. Mix gently by pipetting the solution up and down a few times.
3. Program the thermal cycler according to the manufacturers instructions.
   For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair.
4. Place the tubes in the thermal cycler and start the reaction.

NOTE

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 25 µl.

After primer extension, a sample (10 to 30% of the reaction) can be loaded directly on a gel for analysis.

USER GUIDE

2X PCR Dye Master Mix

Cat. ADPMX02D-100
Qty. 1.25 ml

Content and Storage

Content	Cat	Product	Qty
	ADPMX02D-100	2X PCR Dye Master Mix	1.25 ml

Storage stored in a stable **-20** ℃

Composition of 2X PCR Master Dye Mix

150 mM Tris-HCl pH 8.5,
40 mM (NH4)2S04,
1.5 mM MgCl2,
0.2% Tween 20
0.4 mM dNTPs
0.05 units/µ *Taq* DNA polymerase
Inert red dye and a stabilizer

Description

Taq DNA Polymerase Master Dye Mix is a ready to use 2x reaction mix. Simply add primers, template, and water to successfully carry out primer Extensions and other molecular biology applications. Engine Taq Polymerase, the NH4+ buffer system, , dNTPs and magnesium chloride are conveniently present in the Taq DNA Polymerase Master Dye Mix . An inert red dye and a stabilizer are also present to allow direct loading of the final products onto a gel for analysis. Taq DNA Polymerase Master Dye 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.

Standard Protocol

1. Set up each reaction as follows:

component	Vol./reaction	Final Conc.
2X PCR Dye Master Mix	12.5 µl	1X
Primer A	1 µl (0.5 µl-5 µl)	0.1-1.0 µM
Primer B	1 µl (0.5 µl-5 µl)	0.1-1.0 µM
Distilled Water	Variable	---------
Template DNA	Variable	Genomic DNA : 50 ng (10-500 ng) Plasmid DNA : 0.5 ng (0.1-1 ng) Bacteria DNA : 5 ng (1-10 ng)
TOTAL Volume	25 µl	---------
* Suggested starting conditions; theoretically used conditions in brackets		

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

Three-step PCR program

Cycles	Duration of cycle	Temperature
1	2-5 minutes [a]	95 ˚C
25-35	20-30 seconds [b] 20-40 seconds [c] 30 seconds [d]	95 ˚C 50-65 ˚C 72 ˚C
1	5 minutes	72 ˚C

a.
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.
b.
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 Tm (melting temperature) of the primers used.
c.
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.
d.
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.

USER GUIDE

2X Hi Fi DNA Polymerase MIX

Cat. ADPMX03-100
Qty. 1.25 ml

Content and Storage

Content	Cat	Product	Qty
	ADPMX03-100	2X Hi Fi DNA Polymerase MIX	1.25 ml

Storage stored in a stable **-20** ℃

Description

Hi Fi polymerase synthesizes higher yields of product from genomic DNA, cDNA, bacterial cultures. It is contain a 2.5 hours half life at 96oC and easy to amplify PCR product at G-C rich and secondary structure. 2X Hi Fi Mix is optimized mixture contain of Hi Fi polymerase, reaction buffer, dNTP and enhancer as 2-fold concentration.

2x Hi Fi mix is designed to allow the user for quick ,easy preparation of reaction mixture. The 2x Hi Fi mix can be amplification PCR products up to 10-15 kb and the products can be directly cloning into T-vector.

ARROWTEC

Standard Protocol

Template :

2 x Hi Fi mix is suitable for amplifying targets up to 3 kb from the following templates:

Genomic DNA: 10–200 ng
Plasmid DNA : 1–5 ng
cDNA : ~100 ng starting total RNA

Primers :

Use 0.3 µM per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5 µM per primer may improve yield.

Annealing Temperature :

The annealing temperature is slightly higher than with typical PCR. The optimal annealing temperature should be ~2˚C lower than the Tm of the primers used. A range of 58–68˚C is recommended.

Extension Time :

As little as 30 seconds per kb is suitable for most targets. Use up to 60 seconds per kb for maximum yield.

Standard Protocol

1. Thaw the 2x Hi Fi mix at room temperature. Vortex the 2x Hi Fi mix and then spin it briefly in a micro centrifuge to collect the material in the bottom of the tube.

2. Prepare one of the following reaction mixes on ice:

Step	Volume
2x Hi Fi mix	12.5 µl
Primer1 (20 pmol)	1-2 µl
Primer2 (20 pmol)	1-2 µl
Template	1-10 µl
ddH2O	UP to 25 µl
Total	25 µl

3. If necessary you can scale up your volume

Program the thermal cycler as follows:

Step	Time	Temp	Cycle
Initial denaturation	30 sec - 2 mins	95˚C	1
Denature:	15 sec – 2 mins	95˚C	15-30
Anneal:	15 sec – 2 mins	Tm-5 ˚C	
Extend:	2 mins /1kbp	68-75˚C	
Final extension: 1 - 10 mins 68 - 72°C			

USER GUIDE

2X Hot start Master Mix

Cat. ADPMX04-100
Qty. 1.25 ml

Content and Storage

Content	Cat	Product	Qty
	ADPMX04-100	2X Hot start Master Mix	1.25 ml

Storage stored in a stable **-20** ℃

Description

Hot start Taq DNA Polymerase for qPCR is designed for Real-Time PCR and Hot-start PCR. A special inhibition the reaction at room temperature until after the first denaturation step. This prevents primer-dimers and other artefacts.
2X Hot Start Master mix is optimized mixture contain of Hot Start Taq enzyme, reaction buffer, dNTP and enhancer as 2-fold concentration.
2x Hot Start master mix is designed to allow the user for quick ,easy preparation of reaction mixture. The 2x Hot Start Master mix can be amplification PCR products up to 5 kb and the products can be directly cloning into T-vector.

ARROWTEC

Standard Protocol

1. Set up each reaction as follows:

component	Vol./reaction	Final Conc.
2X Hot Start Master mix	12.5 µl	1X
Primer A	Variable	0.1-1.0 µM
Primer B	Variable	0.1-1.0 µM
Distilled Water	Variable	---------
Template DNA	Variable	Variable
TOTAL Volume	25 µl	---------

2. Mix gently by pipetting the solution up and down a few times.
3. Program the thermal cycler as follows:

Step	Temperature (℃)	Time	Cycle
Initial denaturation	94 – 95	10 mins	1
Denaturation	94 – 95	0.2 – 2 mins	20 – 35
Annealing	50 – 68	0.2 – 2 mins	
Extension	72	1 min / kb	
Final extension	72	1 – 10 mins	1

Standard Protocol

After cycling, maintain the reaction at 4˚C. Samples can be stored at –20˚C until use.

Analyze products using standard agarose gel electrophoresis.

USER GUIDE

2X Hot start Dye Master Mix

Cat. ADPMX04D-100
Qty. 1.25 ml

Content and Storage

Content	Cat	Product	Qty
	ADPMX04D-100	2X Hot start Dye Master Mix	1.25 ml

Storage stored in a stable **-20** ℃

Description

Hot start Taq DNA Polymerase for qPCR is designed for Real-Time PCR and Hot-start PCR. A special inhibition the reaction at room temperature until after the first denaturation step. This prevents primer-dimers and other artefacts.
2X Hot Start Dye Master mix is optimized mixture contain of Hot Start Taq enzyme, reaction buffer, dNTP and enhancer as 2-fold concentration. 2x Hot Start dye master mix is designed to allow the user for quick ,easy preparation of reaction mixture. The 2x Hot Start Dye Master mix can be amplification PCR products up to 5 kb and the products can be directly cloning into T-vector.

ARROWTEC

Standard Protocol

1. Set up each reaction as follows:

component	Vol./reaction	Final Conc.
2X Hot Start Master mix	12.5 µl	1X
Primer A	Variable	0.1-1.0 µM
Primer B	Variable	0.1-1.0 µM
Distilled Water	Variable	---------
Template DNA	Variable	Variable
TOTAL Volume	25 µl	---------

2. Mix gently by pipetting the solution up and down a few times.
3. Program the thermal cycler as follows:

Step	Temperature (℃)	Time	Cycle
Initial denaturation	94 – 95	10 mins	1
Denaturation	94 – 95	0.2 – 2 mins	20 – 35
Annealing	50 – 68	0.2 – 2 mins	
Extension	72	1 min / kb	
Final extension	72	1 – 10 mins	1

Standard Protocol

After cycling, maintain the reaction at 4˚C. Samples can be stored at –20˚C until use.

Analyze products using standard agarose gel electrophoresis.

USER GUIDE

2X Pfu DNA Polymerase MIX

Cat. ADPMX05-100
Qty. 1.25 ml

Content and Storage

Content	Cat	Product	Qty
	ADPMX05-100	2X Pfu DNA Polymerase MIX	1.25 ml

Storage stored in a stable **-20** ℃

Description

2X Pfu DNA Polymerase MIX 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. *Pfu* polymerase, the NH4+ buffer system, dNTPs and magnesium chloride are conveniently present in the Pfu DNA Polymerase Master Mix .
offers several advantages. Set up 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.

ARROWTEC

Standard Protocol

1. Set up each reaction as follows:

component	Vol./reaction	Final Conc.
2X Pfu DNA Polymerase mix	12.5 µl	1X
Primer A	Variable	0.1-1.0 µM
Primer B	Variable	0.1-1.0 µM
Distilled Water	Variable	---------
Template DNA	Variable	Variable
TOTAL Volume	25 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.
4. Place the tubes in the thermal cycler and start the reaction.

Standard Protocol

Program the thermal cycler as follows:

Step	Time	Temp	Cycle
Initial denaturation	30 sec - 2 mins	95˚C	1
Denature:	15 sec – 2 mins	95˚C	15-30
Anneal:	15 sec – 2 mins	Tm-5 ˚C	
Extend:	2 mins /1kbp	68-75˚C	
Final extension: 1 - 10 mins 72°C			

After cycling, maintain the reaction at 4˚C. Samples can be stored at –20˚C until use.

Program the thermal cycler as follows:

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 25 µl.

After primer extension, a sample (10 to 30% of the reaction) can be loaded directly on a gel for analysis.