Twist TrueAmp Polymerase Mix Protocol

The Twist TrueAmp Polymerase Mix is a 2X ready-to-use mix that includes a hot-start enzyme, optimized buffer, dNTPs, and uniformity enhancers for efficient, high-fidelity, and unbiased amplification of next-generation sequencing (NGS) libraries.

The Twist TrueAmp Polymerase Mix hot-start technology features an aptamer, which binds to the polymerase enzyme and prevents degradation of template/primers during room temperature incubations. This aptamer is released from the polymerase at high temperatures during PCR thermal cycling. As a result, even with variable component addition order and on-deck automation setup, the polymerase gives robust and reproducible amplification.

The polymerase in this mix has been engineered to have high fidelity and is able to amplify templates that have GC content ranging from 5% to 95%. It can be used in a wide range of demanding applications, including rare variant detection in challenging cfDNA or FFPE samples, comprehensive analysis in metagenomics and agrigenomics, and highly uniform hybridization capture. It also delivers exceptional performance in routine amplicon, transposase, and whole genome sequencing indexing workflows.

PROTOCOL COMPONENTS

Please read the product packaging and storage recommendations carefully and store components as recommended immediately upon arrival.

CATALOG #	NAME	STORAGE
116471: 16 rxn 116472: 96 rxn	Twist TrueAmp Polymerase Mix	–20°C*

**Twist TrueAmp Polymerase Mix is shipped on dry ice. Upon receipt, store all kit components at -20°C. It is normal if the mix does not freeze completely. When stored and handled as recommended, the product will retain full performance until the expiry date.*

For Research Use Only. *Not intended for use in diagnostic procedures.*

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INTENDED USE

This product is for research use only. This product is not intended for the diagnosis, prevention, or treatment of a disease or condition. Twist Bioscience assumes no liability regarding use of the product for applications in which it is not intended.

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MATERIALS SUPPLIED BY USER

The following materials or their equivalent are required to generate amplified libraries with the Twist TrueAmp Polymerase Mix Protocol.

PRODUCT	SUGGESTED SUPPLIER
REAGENTS AND CONSUMABLES	
Molecular biology grade water	—
Primers (Forward and reverse)	—
Qubit dsDNA Broad Range Quantification Assay	Thermo Fisher Scientific
EQUIPMENT	
Pipettes and tips	—
Benchtop mini centrifuge	—
Benchtop vortexer	—
Thermal cycler with heated lid	—

GENERAL NOTES AND PRECAUTIONS

Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) at all times when performing this protocol.

For best results, read this document before performing the protocol, and follow the instructions provided. Twist cannot guarantee the performance of the Twist TrueAmp Polymerase Mix if modifications are made to the protocol.

Test the compatibility of your thermal cycler and PCR tubes by incubating them at 95°C for up to 5 minutes to ensure the PCR tubes do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

AMPLIFICATION GUIDELINES

PCR TEMPLATE

The Twist TrueAmp Polymerase Mix is compatible with as little as 100 fg of input library mass. Input library template and primers can be stored short-term in molecular biology grade water for simplicity, but low-EDTA Tris buffer is recommended for long-term storage.

PCR ANNEALING

Annealing temperatures and times have been optimized for individual applications and documented in the respective Twist library preparation protocols.

For custom primers, a gradient PCR around a theoretical T_m should be used to empirically determine the annealing temperature.

PCR EXTENSION

Extension temperature at 68°C is recommended. Extension time of 30 seconds per kb is recommended. A 30-second extension is sufficient for short-read libraries. Longer extension times may be used for longer-insert libraries.

PCR CYCLES

Errors and artifacts in PCR increase with higher cycles of PCR. Coverage and uniformity are impacted by overamplification when PCR primers are depleted. For best performance, refer to the table below for starting points regarding optimization of PCR cycle numbers based on input DNA amount. Using the table below, the expected yield is ~1 µg.

INPUT DNA TEMPLATE	PCR CYCLES
10 ng	8
5 ng	9
1 ng	11
100 pg	14
10 pg	17
1 pg	20
100 fg	23

FOR TECHNICAL SUPPORT, CONTACT CUSTOMERSUPPORT@TWISTBIOSCIENCE.COM.

PROTOCOL

This protocol begins with input DNA libraries that are then amplified using various reagents. Afterwards, purification and quality control analysis are performed. This protocol generates amplified libraries in 30 minutes.

Reagents Required

- Input DNA template
- Twist TrueAmp Polymerase Mix
- Forward primer
- Reverse primer
- Molecular Biology Grade Water

Before You Begin

- Thaw all reagents completely and vortex to ensure complete mixing.

1

Program a thermal cycler with the following conditions. **Set the temperature of the heated lid to 105°C.**

STEP		TEMPERATURE	TIME	NUMBER OF CYCLES
1	Initialization	98°C	45 seconds	1
2	Denaturation	98°C	15 seconds	*Variable depending on starting DNA template mass. See Amplification Guidelines section.*
	Annealing	60°C*	30 seconds	
	Extension	68°C	30 seconds**	
3	Final Extension	68°C	1 minute	1
4	Final Hold	4°C	HOLD	–

**Annealing temperature is optimized for both P5/P7 and Twist UDI Primers. For user-supplied primers, optimal annealing temperature may need to be empirically determined (refer to Amplification Guidelines above).*

***Extension time is optimized for 450 bp libraries. For longer fragment libraries, longer extension times may be used.*

2

Calculate the number of reactions required and combine each component according to this table:

COMPONENT	VOLUME PER REACTION
Twist TrueAmp Polymerase Mix	25 µl
Forward primer (100 µM)	1 µl
Reverse primer (100 µM)	1 µl
Input Template DNA	X µl
Water (up to total)	(23-X) µl
Total	**50 µl**

3 Mix the components well by gentle pipetting and then pulse-spin for 2 seconds.

4 Immediately place the reactions in the thermal cycler. Start the program.

5 Once the program is complete, proceed to purification and library quantification using your preferred library preparation workflow.