# AcuGenix™ Hot Start Taq DNA Polymerase



## Product Description

AcuGenix™ Hot Start Taq DNA Polymerase is a hot-start Taq polymerase that completely blocks Taq enzyme activity at room temperature using chemical modification. HS Taq can effectively suppress non-specific amplification caused by primer annealing or primer dimerization at low temperatures, thereby improving the specificity and sensitivity of PCR reactions. It can also be used in a "time release" mode to gradually release enzyme activity during the PCR cycle, further increasing the specificity and sensitivity of amplification for low-copy templates. The main advantages of using this enzyme in fluorescent PCR reactions are high sensitivity, high fluorescence intensity, and high specificity.

## Components

1. 5 U/μL AcuGenix™ Hot Start Taq DNA Polymerase
2. 10×HS Buffer ($Mg^{2+}$ free) (optional)
3. 25 mM $MgCl_2$ (optional)

*10×HS Buffer ($Mg^{2+}$ free) does not contain dNTPs or $Mg^{2+}$. Please add dNTPs and $MgCl_2$ when preparing reaction systems.

## Unit Definition

The amount of enzyme required to incorporate 10 nmol of deoxynucleotide into acid-insoluble material within 30 minutes at 74°C, using activated salmon sperm DNA as the template/primer, is defined as one unit of activity (U).

## Storage Buffer

20 mM Tris-HCl (pH 8.0)，100 mM KCl，0.1 mM EDTA，1 mM DTT，Stabilizer，50% Glycerol.

## Storage

Storage at -20±5℃. Mix thoroughly before use and avoid repeated freeze-thaw cycles.

## Quality Control

1. SDS-PAGE electrophoresis purity no less than 98%
2. Amplification sensitivity, batch-batch difference, and stability.
3. No exogenous nuclease activity, no exogenous endonuclease or exonuclease contamination.

## Prepare the PCR Reaction Mix

| Component | Volume per Reaction | Final Concentration |
|---|---|---|
| 10×HS Buffer ($Mg^{2+}$ free) [1] | 5 μL | 1× |
| dNTPs（10 mM each） | 1 μL | 200 μM |
| 25 mM $MgCl_2$ | 2-8 μL | 1-4 mM |
| 5 U/μL AcuGenix™ Hot Start Taq DNA Polymerase | 0.25-0.5 μL | 1.25-2.5U |
| 25×Primer Mix [2] | 2 μL | 1× |
| Template | —— | <1 μg / Reaction |
| $ddH_2O$ | To 50 μL | —— |

1 . This buffer does not contain dNTPs or $Mg^{2+}$, so they must be added to the reaction system before use.

2. If used for qPCR/qRT-PCR, a fluorescent probe needs to be added to the reaction system. Typically, a final primer concentration of 0.2 μM performs good results; if the reaction performance is poor, adjust the primer concentration within the range of 0.2-1 μM. Typically, probe concentrations are optimized in the range of 0.1-0.3 μM. Combinations of primers

and probes can be tested using gradient experiments to find their optimal combination.

## Thermocycling conditions

| Two-step Method | | | | Three-step Method | | | |
|---|---|---|---|---|---|---|---|
| Procedure | Temp. | Time | Cycle | Procedure | Temp. | Time | Cycle |
| Denaturation | 95℃ | 5~10 min | 1 | Denaturation | 95℃ | 5~10 min | 1 |
| Denaturation | 95℃ | 10~20 s | 35~50 | Denaturation | 95℃ | 10~20 s | 40 |
| Annealing and Elongation | 56~64℃ | 20~60 s | | Annealing and Elongation | 56~64℃ | 10~30 s | |
| | | | | Extension | 72℃ | 10~60 s | |

## Notes

1. Due to differences in temperature control performance among different instruments and its own characteristics, the effect of amplification by five-minute hot start may not be ideal. It is recommended that hot-start time should be optimized within a range of 5~15 min.
2. High specificity and sensitivity make it suitable for multiplex PCR reactions.
3. Performs both 5'-3' polymerase activity and exonuclease activity; no proofreading function or 3'-5' exonuclease activity.
4. Suitable for ordinary PCR, RT-PCR as well as detection methods such as probe method, fluorescence dye method, and gene chip method.
5. PCR products have an A at their 3' end which allows direct T-vector cloning.
6. For primers with low annealing temperatures or amplicons over than 200 bp, three-step cycling is recommende.