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AviHot™

Cat. No.: A10510	250 Units
Cat. No.: A10511	500 Units
Cat. No.: A10512	1000 Units

Store at -20 °C

Component	A10510	A10511	A10512
Enzyme (5U/ µl)	50 µl	100 µl	200 µl
$MgCl_2$ 25 mM	0.5 ml	1 ml	2 x 1ml
10X Buffer	0.5 ml	1 ml	2 x 1ml

Description:

AviHot™ is a mixture of Taq DNA polymerase and a temperature sensitive aptamer-based inhibitor.

The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 40 °C, **but** releases the enzyme during normal PCR cycling conditions.

The aptamer-based hot start mechanism does not require a separate high temperature incubation step to activate the enzyme.

AviHot™ is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer dimers and providing higher specificity of DNA amplification.

The activated **AviHot™** maintains the same functionality as Taq DNA polymerase:

It catalyzes 5' → 3' synthesis of DNA, has no detectable 3' → 5' proofreading exonuclease activity.

Kit storage:

AviHot™ should be stored at **-20 °C**. Under this condition reagents are stable for two years from the date of production.

General Reaction Protocol:

1. Thaw 10X reaction buffer, dNTP mixture.
2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.

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Component	Volume	Final Conc.
10X Reaction Buffer	2 µl	1X
MgCl2 25 mM	1.2 µl	1.5 mM
40 mM dNTPs Mix	0.4 µl	0.2 mM
Upstream Primer (10 pmol/ µl)	1 µl	0.5 pmol/µl
Downstream Primer (10 pmol/ µl)	1 µl	0.5 pmol/µl
Template DNA	Variable	10 fg~1 µg
PCR grade water	Variable	-
AviHot™	0.25 µl	0.065 U/µl
Total Volume	20 µl	-

3. Add templates DNA to the individual PCR tubes or wells containing the master mix.
4. Program the PCR machine according to the program outlined.

Cycle	Time	Temp °C
1	5 min	95
30-35	30 sec	94
	30 sec	57
	30-60 sec	72
1	5 min	72

Notes:

- Extension temperature is between 68 and 72°C. We highly recommend 68 °C for more efficiency of Pars Tous Taq DNA polymerase.
- For PCR products longer than 3~4 Kb, use an extension time of approximately 1 min per Kb DNA.
- A DNA fragment which is amplified by Taq DNA polymerase has an overhang, and it enables you to do cloning by using T-vector

Agarose gel Electrophoresis:

Run the total 5-7 µL of PCR products alongside 3µL DNA marker on a 2% agarose gel containing **Avistain™.**

Disclaimers:

AviHot™ is for **Research Use Only** and should only be used by trained professionals.