Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Hot Start dNTP Mixture each 25mM solution

Product Number: DN32-HS

Shipping and Storage

Stored at -20℃, with a shelf life of 1 year.

Description

Hot Start dNTP Mixture is a dNTP product that has undergone special chemical modifications. Under normal circumstances, Hot Start dNTP Mixture cannot participate in the generation of phosphodiester bonds. Only after heat shock, Hot Start dNTP Mixture will become a regular dNTP and participate in PCR amplification. This product can significantly reduce non-specific amplification caused by non-specific annealing or primer dimers.

Protocol

1. This product is sensitive to temperature and should be avoided from repeated freezing and thawing. It is recommended to pack it in advance before use and thaw it on ice during use.
2. This product begins to show a thermal activation trend at 50-60℃, and when the temperature reaches 95℃, heating for 30-60 seconds can complete the thermal activation.

Note

1. Hot Start dNTP Mixture can be stored at 4℃ for up to 15 days, but it is not recommended to store at room temperature.
2. When using cDNA as a template, it is recommended to use commercially available purification kits to remove unadulterated nucleotides. If the experimental protocol requires the use of unpurified cDNA products, the reaction volume of cDNA synthesized products in the PCR system should not exceed 1/10.
3. In the multiplex reaction of amplifying four or more targets, it is recommended to try adding KCl with a final concentration of up to 100mM to improve the results. The KCl content in the PCR buffer should be considered during the optimization process.

Common problem

Result	Possible reasons	Suggestion
No amplification product or low amplification product yield	Insufficient activation of Hot Start dNTP Mixture during thermal cycling process	Increase the concentration of Hot Start dNTP Mixture to 0.8 mM and the concentration of $MgCl_2$ to 4.0 mM
		Optimize the initial denaturation time to 10 minutes
	The thermal cycle scheme has not been optimized	Increase the extension time, generally the extension time per kb target should be 1-2 minutes;
		Increase the number of thermal cycles, adding 5 cycles each time
		Optimize annealing temperature
	There are issues with the reagents or reaction conditions	Prepare fresh reagents, including reaction buffer and dNTPs
		Verify that the template quality is good and the quantity is sufficient
		Validate primer design to ensure sufficient complementarity with DNA targets
		Optimize $MgCl_2$ concentration (final concentration 2.5 to 4.0 mM)