TaqStart® Antibody Protocol-At-A-Glance

PT1576-2

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I. Introduction

TaqStart Antibody (Cat. Nos. 639250 & 639251) provides an antibody-mediated hot start that enhances the specificity and sensitivity of PCR. This antibody inhibits *Taq* polymerase activity before the onset of thermal cycling, preventing nonspecific amplification and primer-dimer formation. When the reaction temperature is raised, the antibody is quickly inactivated and PCR proceeds. This abbreviated protocol (PT1576-2) is provided for your convenience, but is not intended for first-time users. For additional details, see the TaqStart Antibody User Manual (PT1576-1).

II. General Considerations

A. Storage & Use

Although the supplied TaqStart Antibody stock (>1 µg/ml in buffer containing 50% glycerol) will not freeze at –20°C, it is important to avoid repeated freezing and thawing of diluted TaqStart Antibody by using one of the following two methods:

1. Dilute a portion of the TaqStart Antibody stock with dilution buffer to your desired working concentration, mix it with your *Taq*-based DNA polymerase immediately prior to use, and store at 4°C for ≤3 months. This method is recommended if you plan to use different antibody to polymerase ratios for different experiments (see Section III).
2. Add undiluted TaqStart Antibody directly to your *Taq*-based DNA polymerase, aliquot, and store at –20°C for later use. This method is better if you plan to use the same molar ratio of TaqStart Antibody to polymerase for several experiments (see Section IV).

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B. Choice of Enzymes & Reaction Conditions

TaqStart Antibody is designed to bind and inactivate *Taq*-based DNA polymerases and functions well at a molar ratio of 28:1 (antibody:polymerase). Some *Taq*-based DNA polymerases may require titration to determine their optimum molar ratios relative to TaqStart Antibody—see Section V.B of the User Manual (PT1576-1).

NOTE: The use of DMSO or formamide [or other cosolvents or solutes, (e.g., salts), and pH extremes or other reaction conditions] with TaqStart Antibody is **not recommended** due to interference with antibody function.

III. Protocol: Diluting TaqStart Antibody for Immediate Use in PCR

A. Dilution of TaqStart Antibody

1. Use the supplied dilution buffer to prepare a working dilution of TaqStart Antibody as follows [the resulting 22 µl will be enough to prepare a master mix for 10 (50 µl) PCR reactions]:

4.4 µl	TaqStart Antibody (1.1 µg/µl; 7 µM)
17.6 µl	Dilution Buffer
22.0 µl	TaqStart Antibody (0.22 µg/µl; 1.4 µM)

2. Mix the diluted TaqStart Antibody with your *Taq*-based DNA polymerase at a molar ratio of 28 (molar) parts TaqStart Antibody to 1 (molar) part polymerase, as follows (the resulting 26.4 µl will be enough to prepare 10 (50 µl) PCR reactions, each containing 2.4 µl of the mixture per reaction):

22.0 µl	TaqStart Antibody working dilution (0.22 µg/µl; 1.4 µM)
4.4 µl	*Taq*-based DNA polymerase (5 units/µl or 0.25 µM)
26.4 µl	Total

NOTE: This mixture of TaqStart Antibody and polymerase will freeze at –20°C, but it can be aliquoted and stored at 4°C for ≤3 months for later use. To prepare aliquots of premixed TaqStart Antibody and polymerase that can be stored at–20°C for later use, see Section IV.A.

3. Incubate the TaqStart Antibody/polymerase mixture at room temperature (20–22°C) for 5 min before assembling your PCR reactions. The mixture can be incubated at room temperature for up to 30 min with no adverse effects.

B. Preparation & Use of a PCR Master Mix with Diluted TaqStart Antibody

We recommend preparing a master mix with diluted TaqStart Antibody (Table 1) to minimize tube-to-tube variation in the PCR samples. The following master mix contains enough volume for 10 (50 µl) PCR reactions.

Table 1. PCR Master Mix I

Reagent	Per rxn	For 10 rxns (+10% extra)	Final conc.*
10X PCR reaction buffer	5 µl	55 µl	
20 µM 5' primer	1 µl	11 µl	0.40 µM
20 µM 3' primer	1 µl	11 µl	0.40 µM
dNTP mixture [10 mM each dNTP]	1 µl	11 µl	0.20 µM (each)
PCR-Grade H_2O	37.6 µl	413.6 µl	
Freshly prepared 28:1 mixture of:			
TaqStart Antibody			0.56 µM
+*Taq-based* DNA polymerase	2.4 µl	26.4 µl	0.002 µM
Total volume	48 µl	528 µl	

* Final concentration of components in the reaction mixture, based on a 50 µl final reaction volume.

1. Combine your cDNA or DNA sample with PCR Master Mix I as follows:

48 µl	PCR Master Mix I
2 µl	cDNA or DNA sample
50 µl	Total

2. If necessary, add approximately 50 µl of mineral oil to each tube to prevent evaporation during thermal cycling.
3. Use the DNA thermal cycling program you normally use, with the following considerations:
   a. Using TaqStart Antibody may increase yields or sensitivity, so fewer temperature cycles may be needed to achieve the same yield.
   b. For PCR reactions using extremely low-copy-number target sequences, additional cycles (up to a total of 40–45) may be used to generate enough product to visualize on an agarose gel.

IV. Protocol: Preparing, Storing & Using Aliquots of Premixed TaqStart Antibody & Polymerase

Concentrated TaqStart Antibody may be added directly to an aliquot of *Taq*-based DNA polymerase. The TaqStart Antibody/polymerase mixture may then be aliquoted and stored at –20°C for up to 6 months. This is convenient if you plan to use the same molar ratio of TaqStart Antibody to polymerase for a number of experiments.

NOTE: If the mixture is prepared as described in Section IV.A below, it will not freeze when stored at –20°C, due to the high concentration of glycerol (50%, if the polymerase is also in a storage buffer containing 50% glycerol).

A. Mixing, Aliquoting & Storing TaqStart Antibody/Polymerase

1. Add one volume of TaqStart Antibody to one volume of *Taq*-based DNA polymerase. The example below provides the reagent amounts sufficient for 1 batch of PCR Master Mix II (10 PCR reactions + 10% extra), which is described in Section IV.B. These volumes can be scaled up if you are planning to aliquot and store the premixed TaqStart Antibody/polymerase.

4.4 µl	TaqStart Antibody (1.1 µg/µl; 7 µM)
4.4 µl	*Taq*-based DNA polymerase (5 units/µl; 0.25 µM)
8.8 µl	Total mixture

2. Incubate the TaqStart Antibody/*Taq* DNA polymerase mixture at room temperature (20–22°C) for 5 min before aliquoting and storing or adding it to PCR Master Mix II (Section IV.B). The mixture can be incubated for up to 30 min with no adverse effects.
3. If desired, aliquot the TaqStart Antibody/polymerase mixture for use at a later date. Aliquots prepared this way can be stored at –20°C for up to 3 months.

B. Preparation & Use of a PCR Master Mix with Premixed TaqStart Antibody/Polymerase

We recommend preparing a master mix with the prealiquoted TaqStart Antibody/polymerase mixture (Table 2) to minimize tube-to-tube variation in PCR samples. The following master mix contains enough volume for 10 (50 µl) PCR reactions.

Table 2. PCR Master Mix II

Reagent	Per rxn	For 10 rxns (+10% extra)	Final conc.*
10X PCR reaction buffer	5 µl	55 µl	
20 µM 5' primer	1 µl	11 µl	0.40 µM
20 µM 3' primer	1 µl	11 µl	0.40 µM
dNTP mixture [10 mM each dNTP]	1 µl	11 µl	0.20 µM (each)
PCR-Grade H_2O	39.2 µl	431.2 µl	
Concentrated, premixed			
TaqStart Antibody			0.56 µM
+*Taq* DNA polymerase	0.8 µl	8.8 µl	0.002 µM
Total volume	48 µl	528 µl	

* Final concentration of components in the reaction mixture, based on a 50 µl final reaction volume.

1. Combine your cDNA or DNA sample with PCR Master Mix II as follows:

48 µl	PCR Master Mix II
2 µl	cDNA or DNA sample
50 µl	Total

2. If necessary, add approximately 50 µl of mineral oil to each tube to prevent evaporation during thermal cycling.
3. Begin thermal cycling as described in Section III.B, Step 3.

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