

ClearBand dNTP Mix, 10 mM each

Troubleshooting Guide

1 mL, 5 x 1 mL

Cat No: ETP1, ETP5

Use this guide for common handling and PCR-performance problems encountered with ClearBand dNTP Mix, 10 mM each. The product contains dATP, dCTP, dGTP, and dTTP at 10 mM each in water (pH 7.4). The datasheet specifies storage and composition but does not prescribe a universal working concentration; the final dNTP concentration should therefore be selected for the specific reaction system.

Before You Start - Quick Checks

1. Confirm the product concentration: dATP, dCTP, dGTP, and dTTP are each supplied at 10 mM.
2. For daily/weekly use, store at -20°C.
3. For infrequent use (about 1-2 times per month), store at -80°C.
4. Avoid repeated freeze-thaw cycles; use working aliquots where practical.
5. Mix the solution consistently before pipetting and use accurate low-volume pipetting technique.
6. Calculate the required volume from the concentration of each nucleotide, not only the combined nucleotide concentration.
7. If PCR performance changes, evaluate the complete reaction system rather than assuming the dNTP mix is the only variable.

Problem	Most likely cause	Recommended action
No amplification or very weak PCR product	Incorrect dNTP volume or final concentration; loss of reagent performance after repeated freeze-thaw cycles; or another PCR component/condition is limiting the reaction.	Confirm that the stock is ClearBand dNTP Mix, 10 mM each and recalculate the volume required for the intended PCR formulation. Use a properly stored aliquot and re-check polymerase, MgCl ₂ , primers, template, and cycling conditions. The datasheet does not specify one universal final dNTP concentration, so optimize it for the assay system.
PCR yield decreases after repeated use of the same vial	Reduced performance may reflect repeated freeze-thaw cycles, prolonged time thawed, or another handling/reaction variable. Infrequent use while stored at -20°C is not, by itself, evidence of reagent failure.	Review the vial's actual freeze-thaw history and time spent thawed. Compare with a fresh or low-cycle aliquot while keeping all other PCR variables constant. For future storage, follow the datasheet recommendation: -20°C for daily/weekly use and -80°C for infrequent use.
Results vary between replicate reactions	Pipetting variation, incomplete mixing after thawing, or inconsistent handling of the dNTP stock between reactions.	Thaw under consistent conditions, mix the reagent gently but thoroughly before use, briefly collect the liquid at the bottom of the tube, and prepare a common PCR master mix when appropriate.
Different results after switching from individual dNTPs to the premix	The calculation was based on the concentration of individual nucleotide stocks rather than an equimolar premix containing 10 mM each dATP, dCTP, dGTP, and dTTP.	Recalculate the reaction using the concentration of each nucleotide in the premix (10 mM each). Ensure that the intended final concentration of each nucleotide, not only the total nucleotide concentration, is matched to the assay.
Nonspecific bands or increased background	The dNTP amount may not be optimal for the polymerase/Mg ²⁺ /primer system, or the problem may originate from annealing, template, or other PCR conditions.	Keep the dNTP calculation consistent and optimize the full PCR system. In particular, reassess the dNTP-to-Mg ²⁺ balance together with primer concentration, annealing conditions, template amount, and polymerase settings.
Smearing or poor band definition	Incorrect reagent calculation, variable reaction setup, or suboptimal PCR conditions; repeated freeze-thaw may also reduce consistency over time.	Verify the dNTP volume and final concentration in every reaction, use a fresh properly stored aliquot if handling history is uncertain, and optimize the remaining PCR components and cycling conditions.
PCR performance changes after altering MgCl₂ concentration	dNTPs interact with Mg ²⁺ in PCR, so changing MgCl ₂ can alter the effective reaction balance even when the dNTP stock is unchanged.	When MgCl ₂ is changed, re-optimize the reaction as a complete system rather than changing only one variable. Keep the dNTP concentration fixed during the MgCl ₂ optimization series unless deliberate co-optimization is being performed.

Table continues on next page.

ClearBand dNTP Mix, 10 mM each

Troubleshooting Guide (continued)

Problem	Most likely cause	Recommended action
Unexpected PCR failure after storage or infrequent use	Infrequent use at -20°C is not itself evidence of reagent failure. Repeated/prolonged thawing or other handling history may be responsible.	Review the vial's actual freeze-thaw history and time spent thawed. Compare with a fresh or low-cycle aliquot while keeping all other PCR variables constant. Follow the datasheet storage recommendation for future use.
Unexpected PCR failure despite -80°C storage	Storage at -80°C does not exclude handling-related loss. Repeated thawing, prolonged time thawed, or another PCR variable may be responsible.	Review actual freeze-thaw history and compare with a fresh or low-cycle aliquot. Keep all other PCR components and cycling conditions unchanged during the comparison.
Contamination or false-positive amplification	Repeated opening of reagent tubes, pipetting contamination, or carryover contamination during PCR setup.	Include an NTC (no-template control). If the NTC amplifies, investigate reagent/setup or carryover contamination. Use sterile nuclease-free technique, clean pipetting practices, dedicated PCR setup areas, and fresh aliquots.
Reagent identity or concentration is uncertain	The stock was relabeled, transferred to another tube without complete information, or confused with individual dNTP solutions.	Use clearly labeled aliquots that retain the product identity and concentration: dATP, dCTP, dGTP, and dTTP are each 10 mM. If concentration or identity cannot be confirmed, use a verified stock rather than estimating.
Performance is poor in a non-PCR application	The application may require a different nucleotide concentration or reaction chemistry than the PCR system in which the reagent was previously used.	ClearBand dNTP Mix is intended for PCR and other applications, but the datasheet does not provide application-specific final concentrations. Use the requirements of the specific enzyme/reaction system to determine and optimize the working concentration.

Still having trouble? Verify that the reagent is ClearBand dNTP Mix, 10 mM each and review the actual freeze-thaw history rather than inferring failure from storage frequency alone. Recalculate the intended final concentration of each nucleotide and compare performance with a fresh aliquot while keeping the remaining PCR components constant. Run an NTC if false-positive amplification or contamination is suspected. Because the datasheet does not define one universal working concentration, use the requirements of the polymerase and assay design when optimizing the reaction.