Cormoran® Discovery Taq DNA Polymerase Kit

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Product description

PCR performance depends not only on the DNA polymerase, but also on the surrounding reaction chemistry. Primer sequences, amplicon properties, template type, inhibitors and cycling conditions can all influence which buffer environment provides the best result.

The Cormoran® Discovery Taq DNA Polymerase Kit combines one Cormoran® Taq DNA Polymerase Hot-Start with three different 5× PCR Reaction Buffers, A, B and C. Its aptamer-mediated fast-start hot-start suppresses polymerase activity during reaction set-up without requiring a prolonged activation step. This allows the same assay to be evaluated under three reaction conditions while keeping the polymerase constant.

The kit is intended as a practical starting point for assay development and optimization. Once the preferred buffer has been identified, the corresponding Cormoran® Taq DNA Polymerase Kit with Buffer A, B or C is available in larger formats for routine use.

Kit components

Component	Concentration	Amount
Cormoran® Taq DNA Polymerase Hot-Start	5 U/µL	20 µL
Cormoran® 5× PCR Reaction Buffer A	5×	400 µL
Cormoran® 5× PCR Reaction Buffer B	5×	400 µL
Cormoran® 5× PCR Reaction Buffer C	5×	400 µL

The supplied reagents are sufficient for approx. 100 PCR reactions with a final reaction volume of 20 µL.

Recommended applications

- Establishing new PCR assays and optimizing existing PCR protocols
- Challenging templates or sample matrices and assays affected by PCR inhibitors
- Fast cycling protocols
- Applications requiring improved amplification specificity
- Comparing reaction conditions before assay scale-up

Discovery concept

Use the same primer pair, template, cycling program and polymerase amount with Buffer A, Buffer B and Buffer C. Compare performance side-by-side and select the reaction chemistry that best matches your assay goal.

Reaction set-up

The following 20 µL reaction is recommended as a starting point. Reaction conditions may require assay-specific optimization. For Discovery screening, a real-time PCR readout is recommended whenever possible, for example with a TaqMan probe assay or Cormoran® qPCR GreenDye (20×), because Ct/Cq values allow direct comparison of Buffers A, B and C.

Reagent	Volume for 20 µL	Final concentration
Cormoran® 5× PCR Reaction Buffer A, B or C	4.0 µL	1×
Forward primer, 10 µM	0.8 µL	0.4 µM*
Reverse primer, 10 µM	0.8 µL	0.4 µM*
dNTP mix, 10 mM each	0.5 µL	250 µM each*
Cormoran® Taq DNA Polymerase Hot-Start, 5 U/µL	0.20 µL	1 U/reaction*
Template DNA / sample extract	x µL	assay-dependent
Nuclease-free water	to 20 µL	-
Real-time detection chemistry (recommended for screening)	as required	e.g. TaqMan probe system or Cormoran® qPCR GreenDye (20×)

*Recommended starting point. Typical useful ranges are 0.2-0.5 µM per primer and approximately 0.5-1.25 U polymerase per 20 µL reaction. With a DNA-binding dye such as Cormoran® qPCR GreenDye (20×), a melting-curve analysis can additionally help assess amplification specificity. Melting-curve analysis is generally not applicable to sequence-specific TaqMan probe detection.

Before starting

- Thaw the PCR buffers, dNTPs, primers and template at room temperature or on ice as convenient. Mix thoroughly after thawing and briefly spin down.
- Reaction set-up does not need to be performed on ice. The aptamer-mediated hot-start suppresses polymerase activity during preparation at room temperature. Mix the Cormoran® Taq DNA Polymerase Hot-Start gently before use.
- For the Discovery comparison, prepare three separate master mixes that are identical except for the reaction buffer: one with Buffer A, one with Buffer B and one with Buffer C. Include approximately 5-10% excess volume to compensate for pipetting losses.
- Always include a no-template control. For assay development, appropriate positive and extraction controls are also recommended.
- Use aerosol-resistant filter tips and physically separate reaction set-up from post-PCR analysis to minimize carry-over contamination.

Suggested PCR programs

Step	Cycles	Temperature	Time
Optional initial denaturation	1	95°C	0-2 min
3-step PCR			
Denaturation	30-40	95°C	5-15 s
Annealing	30-40	primer-dependent	15-30 s
Extension	30-40	72°C	15-60 s/kb
2-step PCR			
Denaturation	30-40	95°C	3-15 s
Annealing / extension	30-40	e.g. 60°C*	15-60 s
Optional final extension	1	72°C	2-5 min

The initial 95°C step is optional and is used to denature the sample or target DNA, not to activate the polymerase. The aptamer-mediated hot-start does not require a prolonged activation step. A conventional 3-step PCR can be used, or denaturation can be followed by a combined annealing/extension step for 2-step PCR, for example at 60°C when compatible with the primer or probe assay.

Fast-start aptamer hot-start
The aptamer-mediated inhibition is relieved rapidly during heating. Unlike many chemically modified hot-start systems, no prolonged polymerase activation step is required. The optional 95°C initial denaturation is therefore used only when needed for the sample or target DNA and may be shortened or omitted. For short amplicons, 2-step cycling with denaturation followed by a combined annealing/extension step can be particularly efficient. *Use 60°C only when compatible with the primer/probe set; optimize if required.

How to screen Buffers A, B and C

For a first comparison, prepare three separate master mixes that are otherwise identical and differ only in the 5× PCR Reaction Buffer. Use the same primer/probe concentrations, template input, polymerase amount and cycling program for all three conditions.

- Buffer A: 4.0 µL per 20 µL reaction
- Buffer B: 4.0 µL per 20 µL reaction
- Buffer C: 4.0 µL per 20 µL reaction

Run all three conditions in the same thermal cycling experiment whenever possible. A real-time readout is preferred: compare Ct/Cq values and amplification profiles across Buffers A, B and C. When using Cormoran® qPCR GreenDye (20×) or another DNA-binding dye, also inspect the melting curve to assess amplification specificity.

Evaluation criterion	What to compare
Amplification efficiency	Cq/Ct shift in real-time PCR, product yield in endpoint PCR
Specificity	Single expected product, reduced non-specific products or primer-dimers
Robustness	Consistency across templates, concentrations or sample types
Inhibitor tolerance	Performance with challenging extracts or sample matrices
Fast-cycling suitability	Retention of amplification performance when step times are shortened

Optimization after the first screen

- Select the most promising buffer and then optimize annealing temperature, primer concentration, template input and cycling times if required.
- If amplification is weak, first verify template quality and primer design, then consider increasing cycle number or polymerase amount within the recommended range.
- If non-specific products are observed, increase annealing temperature, reduce primer concentration or template input, and compare whether another Cormoran® buffer provides better specificity.
- For inhibitor-rich samples, evaluate template dilution as well as the three buffer environments. Dilution can reduce inhibitor concentration even when template concentration also decreases.
- For probe-based real-time PCR, compare Ct/Cq values and amplification profiles. For dye-based real-time PCR, additionally use melting-curve analysis where appropriate to check whether the amplified product is specific.

Troubleshooting and assay support

If an assay does not perform as expected, please contact Cormoran® Biotech directly rather than relying on a generic troubleshooting workflow. We are happy to review the assay set-up, primer/probe system, template type, cycling conditions and the results from Buffers A, B and C together with you. Contact us via contact@cormoranbiotech.com or through the Contact Us page at www.cormoranbiotech.com.

Storage and handling

- Store all kit components at -20°C upon receipt.
- Reaction set-up may be performed at room temperature; ice is not required for hot-start performance. Return the reagents to -20°C after use.
- Mix thawed buffers thoroughly before use to ensure homogeneous reaction chemistry.
- Avoid unnecessary repeated freeze-thaw cycles. Aliquoting may be useful for frequently used components.

- When stored correctly, use the reagents until the expiry date stated on the product label or certificate supplied with the product.

Technical notes

- Cormoran® Taq DNA Polymerase Hot-Start is supplied at 5 U/µL.
- The supplied 5× PCR Reaction Buffers are used at a final concentration of 1×.
- The kit does not include primers, dNTPs, template DNA, nuclease-free water or real-time detection chemistry. For Discovery screening, Cormoran® qPCR GreenDye (20×) or a compatible TaqMan probe system can be used for real-time analysis.
- The hot-start function is aptamer-mediated and therefore provides a fast-start system without a prolonged heat-activation step. A compatible DNA-binding dye or fluorescent probe system must be added separately for real-time PCR.
- The three buffers are intended to provide distinct reaction environments. They should be evaluated experimentally for the individual assay rather than assigned a universal performance category.
- Larger pack sizes, bulk supply, customized formulations and special filling formats are available upon request.

Quality and intended use

The Cormoran® Discovery Taq DNA Polymerase Kit is produced in-house by Cormoran® Biotech in Germany. The product is intended for research use. It is not intended for diagnostic procedures unless separately validated and supplied for such use under the applicable regulatory framework.

Technical support and ordering

Product	Cormoran® Discovery Taq DNA Polymerase Kit
Catalogue number	K1201
Manufacturer	Cormoran® Biotech GmbH
Address	Bücklestrasse 3, 78467 Konstanz, Germany
E-mail	contact@cormoranbiotech.com
Website	www.cormoranbiotech.com

One polymerase. Three reaction environments.

Use the Discovery Kit to identify the reaction chemistry that works best for your assay. After selection, the matching Cormoran® Taq DNA Polymerase Kit with Buffer A, B or C can be used for routine workflows and scale-up.

After the discovery screen

When one buffer has shown the desired balance of amplification efficiency, specificity and robustness, transfer the assay to the corresponding Cormoran® Taq DNA Polymerase Kit with Buffer A, B or C for routine work. For larger projects, Cormoran® Biotech can also provide bulk supply, customized formulations and special filling formats.

Support for assay development

If none of the three starting conditions provides satisfactory performance, please contact Cormoran® Biotech directly. Send us the assay format, primer/probe information, template type, amplicon length, cycling conditions and the observed results for Buffers A, B and C. We would be happy to review the data together with you and discuss the most useful next optimization step.

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