For life science research only.
Not for use in diagnostic procedures.

GC-RICH PCR System

Version: 18
Content Version: November 2020

Cat. No. 12 140 306 001 100 U
50 reactions in a final volume of 50 µl

Store kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	purple	GC-RICH PCR System, Enzyme Mix	Enzyme mix in storage buffer: 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20 (v/v), 0.5% Nonidet P-40 (v/v), 50% glycerol (v/v).	1 vial, 50 µl
2	yellow	GC-RICH PCR System, PCR Reaction buffer, 5x conc.	Includes 7.5 mM $MgCl_2$ (final 1.5 mM) and DMSO.	1 vial, 1 ml
3	red	GC-RICH PCR System, GC-RICH resolution solution, 5 M	For amplification of difficult templates.	1 vial, 1 ml
4	blue	GC-RICH PCR System, $MgCl_2$, 25 mM	To adjust final Mg^{2+} concentration.	1 vial, 1 ml
5	colorless	GC-RICH PCR System, Water, PCR Grade	To adjust final reaction volume.	2 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	purple	Enzyme Mix	Store at −15 to −25°C.
2	yellow	PCR Reaction buffer, 5x conc.	Store at −15 to −25°C.
3	red	GC-RICH resolution solution, 5 M	⚠ ***After thawing, crystals are occasionally observed in the solution. Dissolve completely by heating to +37 to +65°C; mix thoroughly.***
4	blue	$MgCl_2$, 25 mM	Store at −15 to −25°C.
5	colorless	Water, PCR Grade	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions
- PCR reaction vessels, such as 0.2 ml thin-walled PCR tubes
- Standard benchtop microcentrifuge
- Thermal block cycler

For PCR

- PCR primers
- Template DNA
- PCR Nucleotide Mix, PCR Grade*

1.4. Application

The GC-RICH PCR System is designed to amplify difficult DNA/cDNA templates up to 5 kb in length including:

- GC-rich targets
- Repetitive sequences
- Uniform amplification of a mixture of nucleic acids.
- With varying GC content, such as multiplex PCR and construction of random libraries.

The GC-RICH PCR System may also be used in standard PCR applications, where it will give better results (higher yield, higher fidelity) than Taq DNA Polymerase alone.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template DNA such as genomic or plasmid DNA, cDNA suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids, use:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).
- Use 10 to 500 ng human genomic DNA or 1 to 100 ng cDNA.

Primers

Use primers at a final concentration of 0.2 to 0.5 µM each. A recommended starting concentration is 0.2 µM each.

Mg2+ Concentration

The optimal Mg concentration is in the range of 1 to 4 mM. The recommended starting concentration is 1.5 mM.

General Considerations

The optimal conditions, including incubation times and temperatures, concentration of Enzyme Mix, template DNA, GC-RICH resolution solution, and magnesium ions depend on the template/primer system and must be determined for each assay.

Titration of GC-RICH resolution solution

1. Using the 1x-concentrated PCR Reaction buffer (Vial 2) may result in the expected PCR products with GC-rich templates. Otherwise, titrate with GC-RICH resolution solution from 0.5 to 2.5 M.
   i *For amplification from GC-rich templates, Master Mix 1 contains GC-RICH resolution solution; Master Mix 2 contains PCR Reaction buffer.*

2. Titrate the GC-RICH resolution solution in steps of 0.5 M in the range of 0.5 to 2 M; if nonspecific bands appear, increase the concentration to 2.5 M.

Final Concentration [M]	0.5	1	1.5	2	2.5
Volume for 50 µl reaction [µl]	5	10	15	20	25

i *GC-RICH resolution solution can also be added to Master Mix 2 instead of Master Mix 1, however, change the volume of Master Mix 1 to 15 µl and Master Mix 2 to 35 µl.*

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

2.2. Protocols

Preparation of PCR master mixes

Prepare two PCR master mixes. Master Mix 2 contains enzyme and reaction buffer; Master Mix 1 contains all other reaction components. This circumvents the need for hot start and avoids that the enzyme interacts with primers or template during the reaction setup.

Preparation of master mix 1

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

2. Prepare a 10x-concentrated solution of each respective PCR primer.
   If you are using, for example, the final concentration of 0.2 µM for each primer, the 10x-concentrated solution would contain a 2 µM concentration of the respective primer.

3. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade	add up to a final volume of 35	–
PCR Grade Nucleotide Mix* (10 mM of each dNTP)	1	200 µM of each dNTP
Forward primer	5	0.2 µM
Reverse primer	5	0.2 µM
Template DNA	variable	10 to 500 ng gDNA 1 to 100 ng cDNA
GC-RICH resolution solution (Vial 3)	variable	0 to 2 M
Final Volume	**35**	

4. Mix and centrifuge briefly.

Preparation of master mix 2

1. Thaw the reagents and store on ice.
   – Briefly vortex and centrifuge all reagents before setting up the reactions.

2. To a sterile reaction tube on ice, add the components in the order listed for each 50 µl reaction:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade	4	–
PCR Reaction buffer, 5x conc. (Vial 2)	10	1x (1.5 mM $MgCl_2$)
Enzyme Mix (Vial 1)	1	2 U/50 µl reaction
Final Volume	**15**	

3. Mix and centrifuge briefly.

PCR protocol

The following thermal profiles are an example. Different thermal cyclers may require different profiles.

1. For each reaction, combine 35 µl Master Mix 1 and 15 µl Master Mix 2 in a thin-walled PCR tube on ice.
   – Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the solution at the bottom of the tube.

   ⚠ ***Start thermal cycling immediately. Do not store the combined reaction mix on ice.***

2. Place your samples in a thermal block cycler and use the thermal profiles below to perform PCR.
   – Run 30 cycles for DNA and 35 cycles for cDNA.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	95[(1)]	3 min	1
Denaturation Annealing Elongation	95 45 – 65 72 or 68	30 sec 30 sec 45 sec/kb, up to 5 kb	10
Denaturation Annealing Elongation	95 45 – 65 72 or 68	30 sec 30 sec 45 sec/kb, up to 5 kb + 5 sec cycle elongation for each successive cycle	20 – 25
Final Elongation	72 or 68	7 min	1

3. Analyze the samples on a 1 to 2% agarose gel.

(1) GC-rich templates require +95°C, 3 minutes in the Pre-Incubation step.

2.3. Parameters

Maximum Fragment Size

Up to 5 kb.

PCR Cloning

The enzyme blend results in more blunt-ended PCR fragments compared to Taq DNA Polymerase. The majority of products still have single A overhangs, therefore TA cloning is the preferred method.

Temperature Optimum

The elongation temperature is +72°C when amplifying fragments up to 3 kb. When amplifying fragments larger than 3 kb, use +66°C for the elongation step.

Volume Activity

2 U/µl

Working Concentration

Enzyme concentration

The optimal enzyme concentration ranges from 0.5 to 5 U per assay. For a standard 50 µl PCR, use 2 U of the enzyme blend per assay.

3. Results

Comparison of the GC-RICH PCR System, Taq DNA Polymerase, and the Expand High Fidelity PCR System

To compare the ability of the GC-RICH PCR System, Taq DNA Polymerase, and the Expand High Fidelity PCR System to amplify GC-rich targets, these systems were used in parallel reactions to amplify a 1.5 kb fragment (GC content, 63%) out of the human TGF-β gene (Figure 1, Panel A) and a 264 bp fragment (GC content, 74%) from the human ApoE gene (Figure 1, Panel B). The experiments demonstrate the effectiveness of the GC-RICH PCR System for amplifying templates with high GC content. The results also demonstrate the enhancing effect that the GC-RICH resolution solution supplied with the system has on the performance of the GC-RICH PCR System (Figure 2).

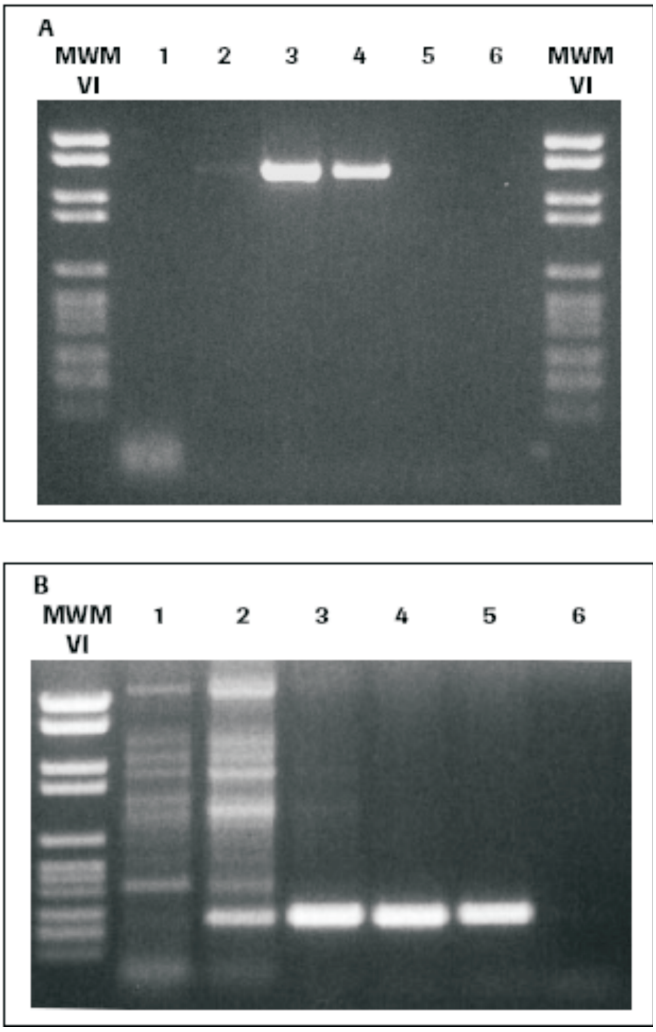

Fig. 1: Amplification of GC-rich templates with the GC-RICH PCR System and Expand High Fidelity PCR System. Two GC-rich DNA templates were amplified for 30 cycles with either of two PCR Systems. Products from each reaction were analyzed on 1% Agarose LE gels.
Panel A shows the products amplified from a 1.5 kb template (GC content, 63%) within the human TGF-β gene.
Panel B shows the products formed from a 264 bp template (GC content, 74%) within the human ApoE gene, DNA Molecular Weight Marker VI, DIG-labeled. The enzyme and reaction supplement used to generate each product (in both panels) were:
Lane 1: Expand High Fidelity PCR System, 2.6 U, no supplement.
Lane 2: GC-RICH PCR System, 2.0 U, without GC-RICH resolution solution.
Lane 3: GC-RICH PCR System, 2.0 U, with 0.5 M GC-RICH resolution solution.
Lane 4: GC-RICH PCR System, 2.0 U, with 1.0 M GC-RICH resolution solution.
Lane 5: GC-RICH PCR System, 2.0 U, with 1.5 M GC-RICH resolution solution.
Lane 6: GC-RICH PCR System, 2.0 U, with 2.0 M GC-RICH resolution solution.

Result: Neither Taq DNA polymerase alone (result not shown) nor the Expand High Fidelity PCR System (Lane 1, Panel A) could amplify the TGF-β template. However, the GC-RICH PCR System successfully amplified the TGF-β fragment when the reaction was supplemented with 0.5 M (Lane 3, Panel A) or 1.0 M GC-RICH resolution solution (Lane 4, panel A).
When the ApoE template was used (Panel B), the Expand High Fidelity PCR System (Lane 1, Panel B) produced only nonspecific products; the system did not amplify the template specifically. The GC-RICH PCR System produced good yields of the specific 264 bp product when the reaction was supplemented with 0.5 to 1.5 M GC-RICH resolution solution (Lanes 3 to 5, Panel B). Taq DNA polymerase alone could not amplify ApoE either (result not shown).

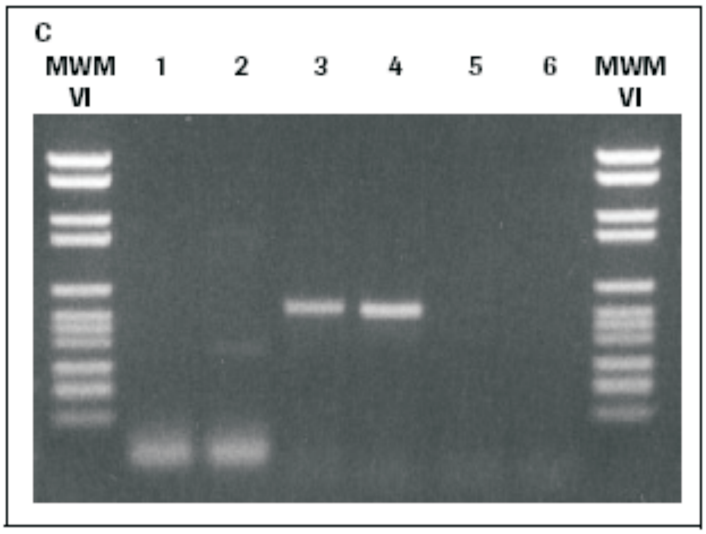

Fig. 2: Amplification of a GC-rich cDNA with the GC-RICH PCR System and Expand High Fidelity PCR System. Total human liver RNA was reverse transcribed with an oligo-(dT) primer and AMV reverse transcriptase. An aliquot of the cDNA was amplified with either of two PCR Systems. The primers in each reaction flank a sequence in the human gene for the IGF II receptor protein. A 500 bp product should be amplified from the cDNA with these primers. The GC content of the amplified target was 62%. All other conditions were as described in Figure 1. The enzyme and reaction supplement used to generate each product were:
Lane 1: Expand High Fidelity PCR System, 2.6 U, no supplement.
Lane 2: GC-RICH PCR System, 2.0 U, without GC-RICH resolution solution.
Lane 3: GC-RICH PCR System, 2.0 U, with 0.5 M GC-RICH resolution solution.
Lane 4: GC-RICH PCR System, 2.0 U, with 1.0 M GC-RICH resolution solution.
Lane 5: GC-RICH PCR System, 2.0 U, with 1.5 M GC-RICH resolution solution.
Lane 6: GC-RICH PCR System, 2.0 U, with 2.0 M GC-RICH resolution solution.
Result: The Expand High Fidelity PCR System produced only a nonspecific product; the system did not amplify the target specifically. However, the GC-RICH PCR System, when supplemented with 0.5 M (Lane 3) or 1.0 M (Lane 4) GC-RICH resolution solution, produced good quantities of the specific 500 bp product. Taq DNA polymerase alone could not amplify the IGF II receptor protein (result not shown).

4. Troubleshooting

Observation	Possible cause	Recommendation
No PCR product detectable.	Difficult templates, such as GC-rich templates.	Titrate GC-RICH resolution solution (Vial 3) 0.5 to 2.5 M in steps of 0.25 M.
	DNA template and primer not optimal.	Check quality and concentration of your template and primer pairs used.
	Cycle conditions not optimal.	Check initial denaturation step; use 3 minutes at +95°C.
		Check annealing temperature and denaturation conditions.
		Increase number of cycles and/or template DNA.
	$MgCl_2$ concentration not optimal.	Titrate $MgCl_2$ concentration (>1.5 mM).
	Enzyme amount not optimal.	Increase amount of enzyme.
High background smear present.	Difficult templates, such as GC-rich templates.	Titrate GC-RICH resolution solution (Vial 3) 0.5 to 2.5 M in steps of 0.25 M.
	Enzyme amount not optimal.	Decrease amount of enzyme.
	DNA template and primer not optimal.	Check quality and concentration of your template and primer pairs used.
	Cycle conditions not optimal.	Check cycle conditions.
	$MgCl_2$ concentration not optimal.	Use 1.5 mM $MgCl_2$.
Nonspecific product present.	Difficult templates, such as GC-rich templates.	Titrate GC-RICH resolution solution (Vial 3) 0.5 to 2.5 M in steps of 0.25 M.
	Cycle conditions not optimal.	Raise annealing temperature.
	Primer design not optimal.	Design alternative primers.

5. Additional Information on this Product

5.1. Test Principle

The GC-RICH PCR System is composed of an enzyme blend of thermostable Taq DNA Polymerase and Tgo DNA Polymerase, a thermostable enzyme with a proofreading (3′→5′ exonuclease) activity. This polymerase mixture by itself outperforms Taq DNA Polymerase in respect to yields, fidelity, and specificity, besides the possibility to amplify fragments up to 5 kb in length. The PCR Reaction buffer in combination with the separately included GC-RICH resolution solution allows to amplify difficult templates, such as GC-rich targets very efficiently.

5.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001

6.4. Trademarks

MAGNA PURE and EXPAND are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany