Accura™ High-Fidelity Polymerase

IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE
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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user, especially the specimens to be amplified, are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support:

Email: techserv@lucigen.com
Phone: (888) 575-9695

Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipping.
Product Description

The Accura High-Fidelity polymerase is a fusion protein, consisting of a DNA binding domain and a unique, thermostable, proofreading polymerase derived from a bacteriophage. When used according to recommendations, Accura High-Fidelity polymerase results in robust amplification of normal, high GC% or otherwise difficult templates. Accura routinely amplifies targets of up to 5 kb; larger targets of up to 10 kb have been achieved with optimization.

Applications for Accura High-Fidelity polymerase include routine cloning, cloning as part of a protein expression workflow, and any PCR reaction where high-fidelity amplification is required (Figure 1). Accura generates amplicons with blunt ends that are compatible with blunt end cloning and Expresso cloning.

Accura is a DNA- and RNA-dependent DNA polymerase intended for DNA amplification. Certain primers may amplify from both DNA and RNA templates therefore care should be taken to remove RNA from samples. Unintended amplification from RNA templates may yield products of unexpected size and sequence. Lucigen recommends the use of a non-specific RNase to fully degrade RNA.

The enzyme is capable of amplifying from many nucleic acid template types, including ssDNA (cDNA) and RNA. As a thermostable DNA polymerase, Accura has a temperature optimum of 72°C on all templates. Below 55°C the enzyme’s activity is greatly reduced. Several factors, including template RNA stability, limit the enzyme’s ability to amplify RNA targets of 400 bases or longer.

Product Designations

<table>
<thead>
<tr>
<th>Product</th>
<th>Kit Size</th>
<th>Catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accura™ High-Fidelity Polymerase</td>
<td>100 Units</td>
<td>30010-1</td>
</tr>
<tr>
<td></td>
<td>500 Units</td>
<td>30010-2</td>
</tr>
</tbody>
</table>
Accura™ High-Fidelity Polymerase

Components and Storage

Store all kits and components at -20°C

The Accura High-Fidelity Polymerase package consists of the following components:

<table>
<thead>
<tr>
<th>Description</th>
<th>Part Number</th>
<th>100 Units</th>
<th>500 Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accura High-Fidelity Polymerase, 2 U/µL</td>
<td>F832528-1</td>
<td>50 µL</td>
<td>5 x 50 µL</td>
</tr>
<tr>
<td>2X Accura HF Reaction Buffer</td>
<td>F882522-1</td>
<td>2 x 1.25 mL</td>
<td>10 x 1.25 mL</td>
</tr>
<tr>
<td>10X Accura GC Reaction Buffer</td>
<td>F882521-1</td>
<td>0.5 mL</td>
<td>5 x 0.5 mL</td>
</tr>
<tr>
<td>Betaine, 5 M</td>
<td>F881901-1</td>
<td>1 mL</td>
<td>5 x 1 mL</td>
</tr>
</tbody>
</table>

Material to be Supplied by the User

- dNTP mix, 2.5mM each
  - (Lucigen recommends PCR Grade dNTPs, catalog number 30030-1)
- Target-specific primers
- Nuclease-free water
- Target RNA or DNA

Standard Setup

Before you start:
1. Always wear gloves while handling the components.
2. Verify sufficient volume of kit components required for planned reactions prior to setup.
3. Pre-warm your thermocycler. If you are using a thermocycler without a heated lid, overlay all reactions with mineral oil.
4. Add target in an area separated from the area where the reaction mix is prepared.
5. Thaw reagents and set up reactions on ice.
6. Reaction setup should be done using good laboratory techniques that minimize cross-contamination.

Accura High-Fidelity polymerase is provided with two separate buffers, the 2X Accura HF buffer and 10X Accura GC Buffer. The 2X Accura HF buffer is the default buffer to be used for most high-fidelity PCR reactions.

In general, users should first attempt to amplify a target using the HF reaction buffer. Amplification of targets that demonstrate less than optimal performance with the HF reaction buffer may improve with the use of the GC reaction buffer and its recommended conditions (see High GC / Difficult Template Setup, page 5). The use of GC buffer or other optimizations for difficult templates may result in lower amplification fidelity.

Standard Reaction Conditions (using 2X Accura HF Buffer):
## Accura™ High-Fidelity Polymerase

<table>
<thead>
<tr>
<th>Volume, µL</th>
<th>Component</th>
<th>Final Concentration or Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2X HF Reaction Buffer</td>
<td>1X</td>
</tr>
<tr>
<td>4</td>
<td>dNTPs (2.5 mM each)(^1)</td>
<td>200µM</td>
</tr>
<tr>
<td>X</td>
<td>Forward Primer</td>
<td>1 µM</td>
</tr>
<tr>
<td>X</td>
<td>Reverse Primer</td>
<td>1 µM</td>
</tr>
<tr>
<td>X</td>
<td>Template DNA(^2)</td>
<td>Plasmid DNA = 100 pg – 30 ng gDNA = 10 ng – 100 ng</td>
</tr>
<tr>
<td>0.5</td>
<td>Accura High-Fidelity Polymerase, 2 U/µL</td>
<td>1U</td>
</tr>
<tr>
<td>X</td>
<td>Nuclease-free H₂O</td>
<td>---</td>
</tr>
<tr>
<td>50</td>
<td>Total volume</td>
<td></td>
</tr>
</tbody>
</table>

1. Most reactions will work optimally with a dNTP concentration of 200 µM. However, for longer targets (5 kb or greater), high GC templates, or sequences with difficult structure, increasing dNTP concentration to 400 µM may result in better amplification.

2. Lucigen recommends starting with the stated range. If the reaction is unsuccessful, additional copies of template may be needed. Final quantity of input DNA will depend on target and copy number.

### Standard Cycling Conditions (2X Accura HF Buffer):

The following general program is recommended for thermal cyclers:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initial Denaturation</td>
<td>94°C(^1)</td>
<td>30 seconds</td>
</tr>
<tr>
<td>2. Denaturation</td>
<td>94°C(^1)</td>
<td>15 seconds</td>
</tr>
<tr>
<td>3. Annealing</td>
<td>60°C(^2)</td>
<td>15 seconds</td>
</tr>
<tr>
<td>4. Extension</td>
<td>72°C</td>
<td>60 sec /kb(^3)</td>
</tr>
<tr>
<td>5. Repeat steps 2-4</td>
<td></td>
<td>20 – 30 total cycles(^4)</td>
</tr>
<tr>
<td>6. Final extension</td>
<td>72°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>7. Hold</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

1. Accura in HF Buffer requires a 94°C denaturation temperature. Lucigen does not recommend the use of denaturation temperatures higher than 94°C using Accura in HF Buffer.

2. Annealing temperature may be adjusted to accommodate primers of various melting temperatures. See Additional Amplification Guidelines for more information.

3. Extension times of 30 seconds per kb have proven suitable for some smaller targets.

4. 20-25 cycles are sufficient for most reactions. Additional cycles are only recommended for weakly performing reactions or long templates. Excessive cycling may result in non-specific amplification.

### High GC / Difficult Template Setup

The 10X Accura GC reaction buffer is intended for use with templates that have high GC content or are difficult to amplify for other reasons, such as a tendency to form secondary structure. Amplification of targets that demonstrate less than optimal performance with the HF reaction buffer may improve with the use of the GC reaction buffer and the addition of Betaine. Amplification using GC buffer and other optimizations may result in lower fidelity as compared with using HF buffer.

### High GC / Difficult Template Reaction Conditions (10X Accura GC Buffer):
**Accura™ High-Fidelity Polymerase**

<table>
<thead>
<tr>
<th>Volume, µL</th>
<th>Component</th>
<th>Final Concentration or Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10X GC Reaction Buffer</td>
<td>1X</td>
</tr>
<tr>
<td>20-25</td>
<td>5 M Betaine</td>
<td>2.0-2.5 M</td>
</tr>
<tr>
<td>8</td>
<td>dNTPs (2.5 mM each)</td>
<td>400 µM</td>
</tr>
<tr>
<td>X</td>
<td>Forward Primer</td>
<td>1 µM</td>
</tr>
<tr>
<td>X</td>
<td>Reverse Primer</td>
<td>1 µM</td>
</tr>
<tr>
<td>X</td>
<td>Template DNA¹</td>
<td>Plasmid DNA = 100 pg – 30 ng</td>
</tr>
<tr>
<td></td>
<td>gDNA = 10 ng – 100 ng</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>Accura High-Fidelity Polymerase, 2U/µL</td>
<td>1U</td>
</tr>
<tr>
<td>X</td>
<td>Nuclease-free H₂O</td>
<td>---</td>
</tr>
<tr>
<td>50</td>
<td>Total volume</td>
<td></td>
</tr>
</tbody>
</table>

1. Lucigen recommends starting with the stated range. If the reaction is unsuccessful, additional copies of template may be needed. Final quantity of input DNA will depend on target and copy number.

**Note:** If you plan to include additional reagents, such as those needed for quantitation of the reaction product or study of reaction kinetics, reduce the amount of nuclease-free water used to maintain the 50 µL total reaction volume.

**High GC / Difficult Template Cycling Conditions (10X Accura GC Buffer):**

The following general program is recommended for thermal cyclers:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Initial Denaturation</td>
<td>98°C¹</td>
<td>2 minutes</td>
</tr>
<tr>
<td>2. Denaturation</td>
<td>98°C¹</td>
<td>15 seconds</td>
</tr>
<tr>
<td>3. Annealing</td>
<td>60°C²</td>
<td>30 seconds</td>
</tr>
<tr>
<td>4. Extension</td>
<td>72°C</td>
<td>60 sec /kb</td>
</tr>
<tr>
<td>5. Repeat steps 2-4</td>
<td></td>
<td>25 – 35 total cycles³</td>
</tr>
<tr>
<td>6. Final extension</td>
<td>72°C</td>
<td>10 minutes</td>
</tr>
<tr>
<td>7. Hold</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

1. Reactions using GC Buffer must be cycled at 98°C.
2. Annealing temperature may be adjusted to accommodate primers of various melting temperatures. See *Additional Amplification Guidelines* for more information.
3. 25-35 cycles is sufficient for many reactions. Additional cycles are only recommended for sub-optimally performing reactions or long templates. Excessive cycling may result in off-target amplification.

**Other optimization notes**

To reduce or prevent primer dimers or other spurious amplification, avoid excessive concentrations of primer, enzyme or betaine, and do not use more PCR cycles than necessary. Primer dimers may be reduced by using a lower concentration of primers than specified under the reaction conditions.

In certain suboptimal conditions, users may observe high molecular weight amplification product when visualizing the PCR product on a gel. This is usually seen as a band near or in the loading well of the gel.

Use good laboratory practices for amplification, such as:
- Changing gloves and pipet tips often
Accura™ High-Fidelity Polymerase

- Using nuclease-free reagents
- Performing reaction set-up and amplification in different areas to avoid environmental contamination

**Additional Amplification Guidelines**

1. **Cold Reaction Set-Up**
Reactions using Accura High-Fidelity polymerase yield best results when set up on ice and maintained at 4°C prior to amplification. The polymerase has residual activity at temperatures above 4°C that can cause non-specific background amplification. Add primers or polymerase to reactions just prior to incubation/cycling.

2. **Template Preparation**
Most routine methods of template purification are sufficient (e.g. phenol/chloroform or guanidine/silica-based methods). However, trace amounts of purification agents (phenol, EDTA, Proteinase K, ethanol, etc.) may inhibit amplification. It is preferred that the nucleic template be dissolved in water or EDTA-free buffer rather than TE following purification. If TE is required, formulation with 0.1 mM EDTA will give best results.

3. **Reaction Overlay**
A thermal cycler with a heated lid is ideal to prevent evaporation of the reaction mix. If no such lid is available, the reaction mixture can be overlaid with one-half reaction volume of PCR-grade mineral oil.

4. **Denaturation Temperature**
Accura High-Fidelity polymerase will amplify most routine DNA templates using a denaturation temperature of 94°C. For GC-rich templates, with GC content up to 75%, a denaturation temperature of 98°C is required.

5. **Primer Design**
Traditional PCR primer design best practices should be followed. These include:

- Avoid extreme G/C or A/T content if possible
- Primers should be at least 25 bases long.
- Avoid multiple G or C bases at the 3’ end of the primer, because this may promote nonspecific binding.
- Design primers that are free of inverted repeats which may form hairpins or other inhibitory secondary structures.
- The 3’ end of the primers should not be complementary to themselves or any other primer in the reaction.
- Primer melting temperatures should differ by no more than 5°C.
- For degenerate primers, at least three conservative bases must be located within the 3’ end of the primer.

Using a primer design tool such as those listed below is strongly recommended.

- Primer3: [http://biotools.umassmed.edu/bioapps/primer3_www.cqi](http://biotools.umassmed.edu/bioapps/primer3_www.cqi)
6. Annealing Temperature
An annealing temperature of 60°C is recommended for most reactions. For best results, the annealing temperatures should be adjusted to be 2°C lower than the lowest melting temperature of your primers.

7. Minimizing non-specific amplification
When using very low amounts of template, or a high concentration of primers, users may observe a high molecular weight amplification product. This can sometimes be observed as DNA that appears to remain in the well of an agarose gel. The use of a higher concentration of dNTPs or additives such as BSA may reduce this phenomenon.
Appendix A: Quality Control Assays

**Activity Assay**
Polymerase activity is assayed at 72°C with 0.2 mM each of dATP, dGTP, dTTP, dCTP (mix of unlabeled and [33P] dCTP); 10 µg activated calf thymus DNA, and 0.1 mg/mL BSA.

**Absence of Endonuclease**
Accura Buffers are tested to be free of detectable endonuclease or nicking activity. One µg of supercoiled plasmid DNA is incubated with buffer for 16 hours at 70°C. Agarose gel electrophoresis shows no alteration in mobility, consistent with endonuclease or nicking activity.

**Absence of Exonuclease**
Accura Buffers are tested to be free of contaminating exonuclease activity by incubating with 1 µg of Hind III-digested lambda DNA at 70°C for 16 hours. Agarose gel electrophoresis shows no alteration in mobility, consistent with exonuclease activity.

**Absence of Ribonuclease**
Accura Polymerase is tested to be free of contaminating RNAse activity by incubating with a fluorogenic RNAse substrate for 1 hour at 37°C. No increase in assay fluorescence above background is detected.
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