



# IG® RPA Master Mix

## Manual

|                     |              |               |               |
|---------------------|--------------|---------------|---------------|
| <b>Catalog #</b>    | <b>3545</b>  | <b>3547</b>   | <b>3549</b>   |
| <b>Package Size</b> | 25 reactions | 100 reactions | 500 reactions |
| <b>Volume</b>       | 400 µl       | 1.6 ml        | 8 ml          |



### Important!

#### **-20°C Storage Required**

- \* Immediately inspect packages
- \* Freeze upon receipt



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## Description:

**IG® RPA Master Mix** is a ready to use premix which contains T4 UvsX, UvxY, gp32, Bsu DNA polymerase, and stabilizers with an optimized reaction solution. It simplifies and quickens the setup for your RPA reaction. The proprietary IG® RPA Master Mix offers two key advantages over other RPA products: 1) More robust amplification at shorter times and 2) Lower background amplification of off-target products meaning IG® RPA Master Mix has more specific amplification of the primary target.

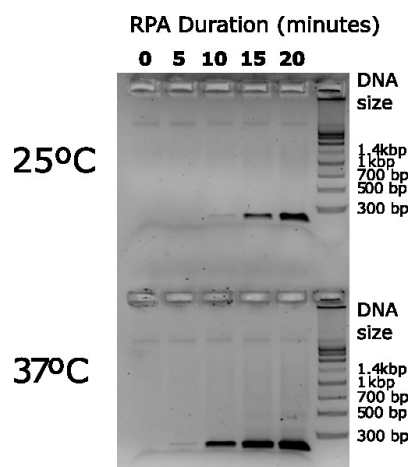


Figure 1: IG RPA Master Mix amplified DNA in 15 minutes at 25°C, in 10 minutes at 37°C.

## Benefits:

- Highly selective and sensitive isothermal amplification technique.
- Works at 25–37°C. No thermocycler needed. Alternative to PCR.
- Speed and sensitivity. Excellent for rapid molecular and agricultural tests.
- Flexible endpoint detection compatibility, (e.g. lateral flow, real-time fluorescence).
- Simplify your workflow. Amplify your results.

## Applications:

### 1. Healthcare and Infectious Disease Diagnostics

Rapid detection of pathogens such as SARS-CoV-2, HIV, influenza, and tuberculosis.  
Decentralized testing in clinics, emergency settings, and resource-limited areas.

### 2. Food Safety

On-site detection of foodborne pathogens (e.g., *Salmonella*, *E. coli*).  
Verification of allergen contamination and quality control.

### **3. Agriculture**

Early detection of plant pathogens and crop diseases.  
Monitoring livestock infections.

### **4. Environmental Monitoring**

Detection of microbial contamination in water supplies.  
Biosurveillance of emerging pathogens in ecosystems.

### **5. Biodefense and Security**

Rapid field-deployable assays for detection of biothreat agents.

## **Specifications:**

Enzymes: Nuclease free, >95% purity by Coomassie stained polyacrylamide gel analysis.

Format: Tubes

Shipping Condition: Dry ice & frozen gel packs

## **Product Components:**

- IG® RPA Master Mix
- RPA Reaction Starter

## **Storage:**

- IG® RPA Master Mix: -20 °C
- RPA Reaction Starter: -20 °C or 4 °C

## **Quality Control:**

All enzymes thoroughly tested for activity and purity.

All component proteins of the Enzyme Mixture are free from detectable nuclease activities.

RPA activity of each lot is validated to be consistent with prior IG® lots and to be equal to or better than major competitors in the market.

Product quality management system is ISO 13485 certified.

## General Guidelines:

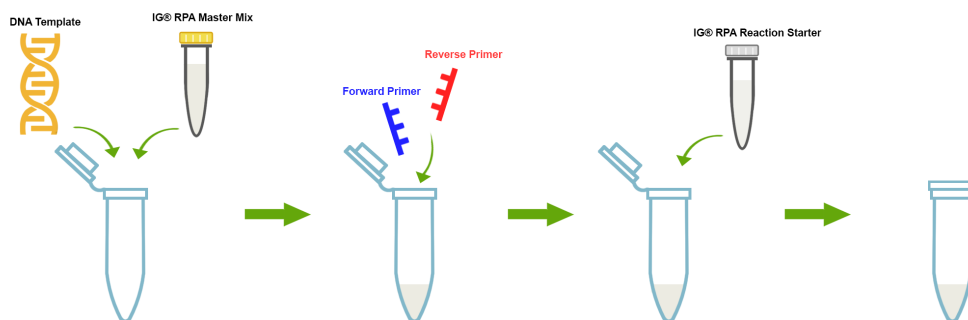
- The master mix may have a pearl-colored appearance, which is normal. It typically clears during RPA.
- Setting up the reaction on ice is recommended for consistent performance.
- Mixing is very important to ensure a homogeneous reaction. Mix the reagents well by pipetting before using. Mix by pipetting half of the total volume 10 times.
- Low copy DNA targets may require longer incubation times, warming to 37°C, and/or higher template concentration.
- Primers are optimally 25-35 BP in length and do not form either homo- or hetero-dimers. Primers should be checked to minimize secondary structures, e.g., hairpins.

## Protocol:

1. Add the DNA template and IG RPA Master Mix in a tube on ice; mix by pipetting.
2. Add the forward and reverse primers.
3. Add the IG RPA Reaction Starter to initiate the reaction. Prepare per reaction mix according to the following table in order:

| Component                  | Volume         |
|----------------------------|----------------|
| DNA Template (1 fg-100 ng) | 1.0 µL         |
| IG® RPA Master Mix         | 16.0 µL        |
| Primer F (10 µM)           | 1.0 µL         |
| Primer R (10 µM)           | 1.0 µL         |
| RPA Reaction Starter       | 1.0 µL         |
| <b>TOTAL</b>               | <b>20.0 µL</b> |

4. Mix half of the total volume 10 times by pipetting, then pulse spin the reaction tube for 1 second.
5. Incubate the tube at 37°C for 5 – 30 minutes or at 25°C for 10 – 45 minutes.



6. (Optional) Purify the DNA via ethanol precipitation. Analyze the DNA by gel electrophoresis on a 1% or 2% agarose gel.

## Q&A

Q: How long should the RPA incubate?

A: The IG® RPA Master Mix is a very sensitive assay and extending the time may enhance the signal for low DNA copy targets (under 1000 copies). The table below is a flexible guide to help with designing applications:

| RPA Reaction Temperature | # copies of DNA<br>High Copy DNA    | Time to detection:<br>electrophoresis | # copies of DNA<br>Low Copy DNA | Time to detection:<br>electrophoresis |
|--------------------------|-------------------------------------|---------------------------------------|---------------------------------|---------------------------------------|
| 25°C                     | 10 <sup>7</sup><br>(<100 picograms) | <15 minutes                           | <1000<br>(<1 femtogram)         | <45 minutes                           |
| 37°C                     |                                     | <10 minutes                           |                                 | <30 minutes                           |

Q: How important is mixing?

A: The reaction mixture is more viscous than saline solutions. It is advised to mix in the DNA template well by pipetting 10 times , then mix 10 times again after the RPA reaction starter is added.

Q: Should the reagents be kept cold?

A: For best results, set up the RPA reactions on ice and then begin your reaction by moving the reaction vial to ambient temperature or an incubator. Once the start solution is added, the reaction rate is temperature dependent and the reaction is fast at ambient temperature, but very slow on ice. Store any unused IG® RPA Master Mix in a freezer at –20°C.

## Related Products

1. T4 UvsX Recombinase (Cat.# 3562)
2. T4 gp32 Protein (Cat.# 3515)
3. T4 UvsY Protein (Cat.# 3572)
4. Bsu DNA Polymerase (Cat.# 3585)
5. Sau DNA Polymerase (Cat.# 3595)
6. Exonuclease III (Cat.# 3415)
7. Exonuclease IV (Nfo) (Cat.# 3425)

## Ordering Information

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.  
Email: **sales@intactgenomics.com**  
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.

## References

1. Cromie GA, Connelly JC, Leach DR (2001) Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. *Mol Cell* 8: 1163–1174
2. Michel B, Grompone G, Flores MJ, Bidnenko V (2004) Multiple pathways process stalled replication forks. *Proc Natl Acad Sci U S A* 101: 12783–12788
3. Liu J, Ehmsen KT, Heyer WD, Morrical SW (2011) Presynaptic filament dynamics in homologous recombination and DNA repair. *Crit Rev Biochem Mol Biol* 46: 240–270

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Our hours are Monday - Friday, 8 AM to 5 PM, U.S. central standard time (CST).

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