Intact Genomics®

# IG® Recombinase Polymerase Amplification Kit Version 2 (IG®RPA Kit v2)

## Manual

| Catalog # | 3533 | 3534 | 3536 |
|---|---|---|---|
| Package Size | 25 Reactions | 100 Reactions | 500 Reactions |

## Important!

**-20°C Storage Required**

* Immediately inspect packages
* Freeze upon receipt

*visit us online for more products & custom services*

**Intact Genomics, Inc.**

Intact Genomics®

# IG® RPA Kit v2 Manual

## Table of Contents


**Product Description & Benefits** ........................................................ 3

**Components and Storage** ........................................................ 3

**RPA Reaction Protocol** ........................................................ 4

**RPA Reaction with Positive Control** ........................................................ 4

**RPA Technology Applications & Challenges** ........................................................ 5

**Quality Control**........................................................ 6

**References**........................................................ 6

**Related Products** ........................................................ 6

**Ordering Information**........................................................ 6

**IG Enzyme Concentration** ........................................................ 7

**Technical Support** ........................................................ 8

## Description:

The new improved IG® Recombinase Polymerase Amplification Version 2 Kit (IG® RPA v2) helps researchers who seek the advantages of isothermal DNA amplification to achieve faster, more discernible results. IG® RPA v2 offers two key advantages over other kits:

- More robust amplification.

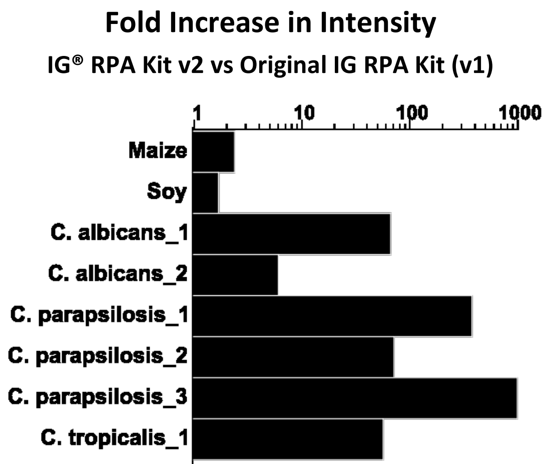



*Figure 1: The ratios of IG® RPA kit v2 to RPA original formula using gel densitometry to quantify RPA band intensity. Amplicon band intensities of RPA Kit v2 are between 1.7x to 1000x more robust than the intensity of the original RPA mix using genomic DNA as targets for various primer sets.*

- More specific amplification of the primary target. Reduces background amplification of off-target sites.

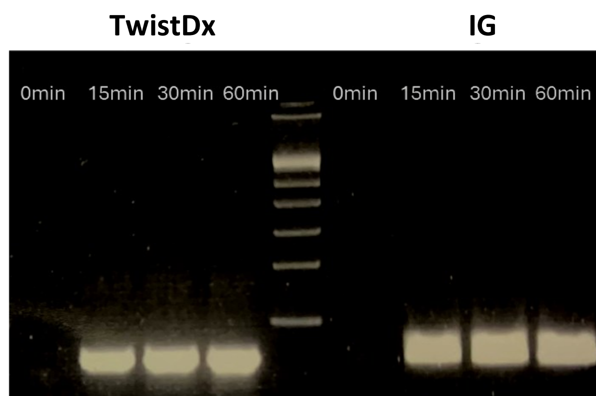



*Figure 2: Comparing with TwistDx kit, IG® RPA Kit v2 showed more robust amplification, more specific amplification of the primary target and less off-target site background after 37°C incubation.*

## Kit Includes:

- 4x IG® RPA Enzyme Mixture v2
- 2.5x IG® RPA Reaction Master Mix v2
- 280 mM Magnesium Acetate (MgOAc)
- RPA Positive Control (Primers and Template)
- Nuclease-free Water

## Storage Temperature: -20°C

## RPA Reaction Protocol:

1. Thaw IG® RPA Enzyme Mixture, Reaction Master Mix, Nuclease-free Water and DNA Primers.
2. Due to the viscosity of the Reaction Master Mix, it is recommended to **mix the DNA primers and RPA Enzyme Mixture on ice first**. Then, add the template and RPA Reaction Master Mix and mix by pipetting 10 times.
3. Prepare a reaction mix according to the following table:

| Component | Volume |
|---|---|
| Primer F (10 µM) | 1 µL |
| Primer R (10 µM) | 1 µL |
| 4x IG® RPA Enzyme Mixture v2 | 5 µL |
| DNA Template (1 fg-100 ng) | 1 µL |
| Nuclease-free Water | 3 µL |
| 2.5x IG® RPA Reaction Master Mix v2 | 8 µL |

4. To initiate the reaction, add 1 µL of MgOAc per reaction, mix thoroughly, and pulse spin the reaction tube briefly. **RPA reactions start as soon as MgOAc is added**.
5. Incubate the tube at 37°C for 5-40 minutes. The reaction may also be performed at ambient temperature (~24°C) . However, we recommend increasing the incubation time to a minimum of 30 minutes.
6. Purify the DNA via ethanol precipitation(or the user's preferred method). Analyze the DNA by gel electrophoresis on a 1% agarose gel.

## RPA Reaction with IG RPA Positive Control

1. Mix Positive Control with IG® RPA Enzyme Mixture thoroughly, then add Reaction Master Mix.
2. Prepare a reaction mix according to the following table:

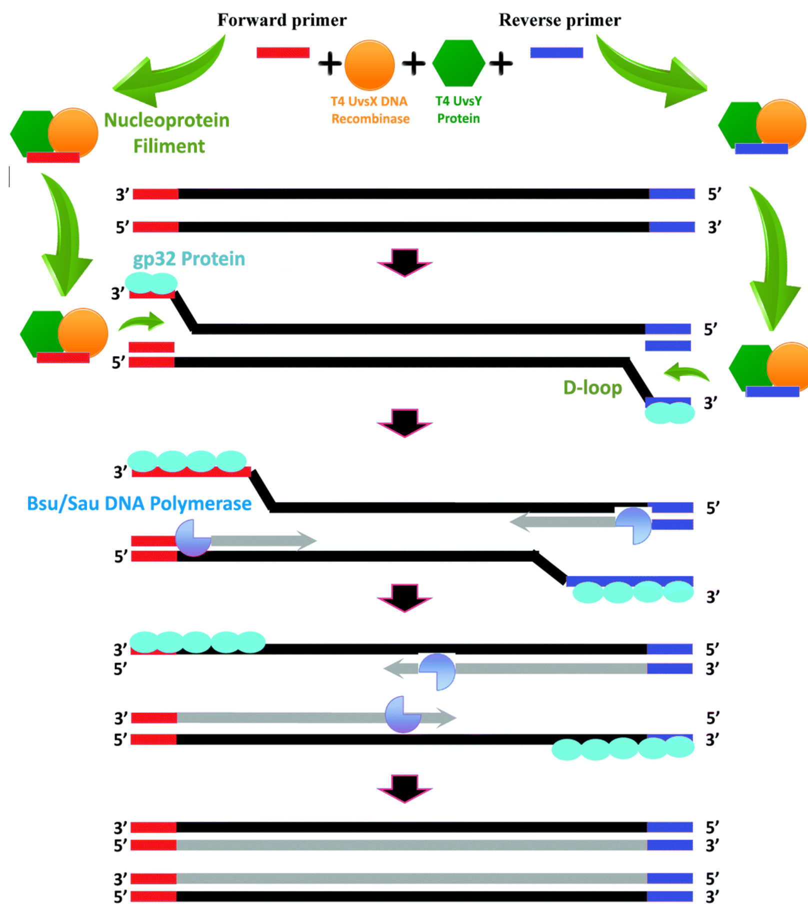
| Component | Volume |
|---|---|
| RPA Positive Control (Primers and Template) | 3 µL |
| 4x IG® RPA Enzyme Mixture v2 | 5 µL |
| 2.5x IG® RPA Reaction Master Mix v2 | 10 µL |

3. To initiate the reaction, add 1 µL of MgOAc per reaction, mix thoroughly, and pulse spin the reaction tube briefly. **RPA reactions start as soon as MgOAc is added.**
4. Incubate the tube at 37°C for 15 minutes. The reaction may also be performed at ambient temperature (~24°C) . However, we recommend increasing the incubation time to a minimum of 30 minutes.
5. Purify the DNA via ethanol precipitation (or the user's preferred method). Analyze the DNA by gel electrophoresis on a 1% agarose gel.

## Recombinase Polymerase Amplification Technology

**IG® Recombinase Polymerase Amplification Kit Version 2 (IG® RPA V2)** amplifies DNA at a single and constant temperature (25 - 42°C) using a recombinase (e.g. UvsX), primers, a single-stranded DNA binding protein (SSB), and a strand displacing DNA polymerase. T4 UvsX is used in combination with its accessory protein, UvsY. The recombinase interacts with the primers to form nucleoprotein filament. This complex can bind with homologous double-stranded DNA through a strand exchange[1-3]. After the exchange, a single-stranded binding protein, T4 gp32, stabilizes the displaced strand. Finally, Bsu/Sau DNA polymerase extends the DNA from the primers, creating a new complete copy of the template, and amplification can continue like in Polymerase Chain Reaction (PCR).

### Schematic of Recombinase Polymerase Amplification (RPA)




©2018 Intact Genomics

## Applications and Benefits:

- Highly selective and sensitive isothermal amplification technique.
- Alternative to PCR. No thermocycler or other heavy equipment needed.
- Speed and sensitivity. Excellent for rapid point-of-care and on-site testing.
- No DNA pretreatment required.
- Flexible endpoint detection compatibility (e.g. lateral flow, real-time fluorescence).

## Challenges:

Recombinase Polymerase Amplification (RPA) is a popular isothermal amplification technique used in molecular diagnostics. However, like any new technology, it faces certain challenges. Intact Genomics has been working in the RPA field for many years and our scientists have technical expertise to support the scientists and their research with this promising technology. Contact us for tech support and collaboration opportunities. We are committed to continuous innovation and optimization and we are likely to address many of these issues in our studies.

## Quality Control:

- 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.

## 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


## Related Products:

1. FastAmp® qRPA SYBR kit (Cat.# 3612, 3614)
2. T4 UvsX Recombinase (Cat.# 3562) , Glycerol-free T4 UvsX DNA Recombinase (Cat.# 3562GF)
3. T4 gp32 Protein (Cat.# 3515), Glycerol-free T4 gp32 Protein (Cat.# 3513GF)
4. T4 UvsY Protein (Cat.# 3572), Glycerol-free T4 UvsY Protein (Cat.# 3572GF)
5. Bsu DNA Polymerase (Cat.# 3585), Glycerol-free Bsu (Cat.# 3585GF)
6. Exonuclease IV (Nfo) (Cat.# 3425)

## Ordering Information:

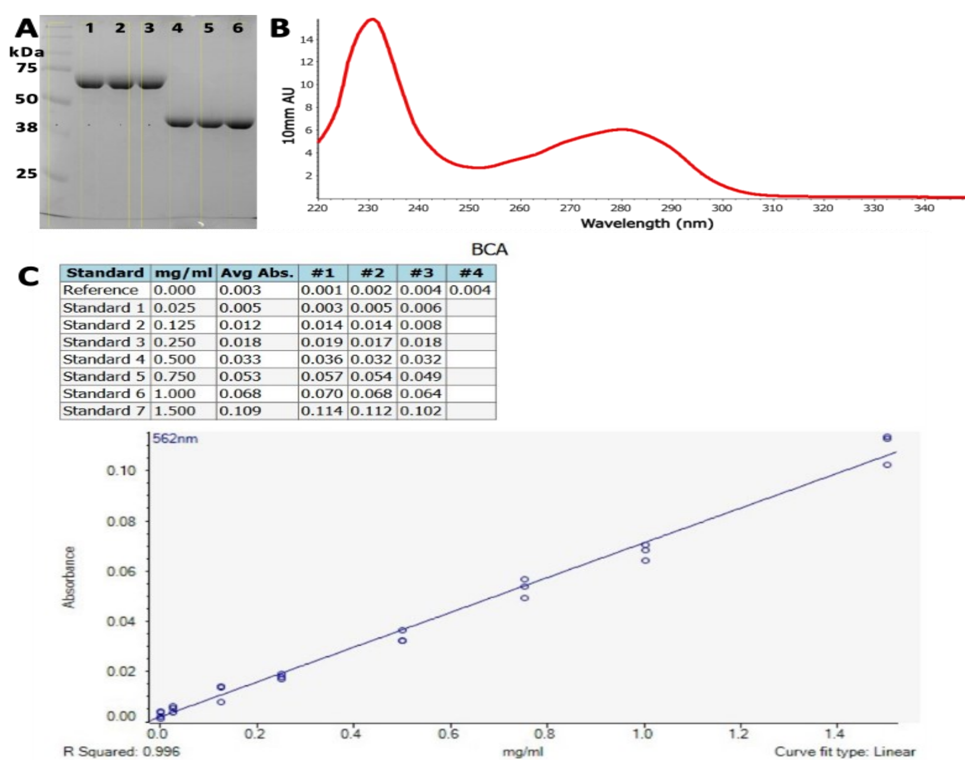
- Order online within the USA. Place orders on www.intactgenomics.com using our secure Shopping Cart.
- Order by email, phone, or fax.
  **Email:** sales@intactgenomics.com
  **Phone:** (314) 942-3655 | **Toll-free :** 855-835-7172 | **Fax:** (314) 942-3656
- Order from our distributors.

## IG® Enzyme Concentration:

Intact Genomics (IG®) uses orthogonal, 3-part approaches to determine the enzyme concentration to provide you with consistent and reliable enzymes for your needs. The quantity of a protein sample is assessed using densitometry with polyacrylamide gel electrophoresis (PAGE), UV absorbance spectra of native protein, and using a protein standard assay such as bicinchoninic acid assay (BCA) using bovine serum albumin (BSA) as a standard (Figure 3).

**Why does IG use all three approaches?**

1. Each method above has limitations. The limitations include experimental noise, accuracy, and susceptibility to buffer and/or enzyme conditions.
2. Each enzyme has unique physical properties that make a single approach to analyzing proteins a challenge. Each enzyme has a different protein sequence, different requirements to be stable in solution, and different requirements to retain its maximal activity. These differences can interfere with or convolute results, especially when compared to other enzymes. When used together, however, each method provides the scientist with independent measures of both enzyme and buffer purity and quality.




| Standard | mg/ml | Avg Abs. | #1 | #2 | #3 | #4 |
|---|---|---|---|---|---|---|
| Reference | 0.000 | 0.003 | 0.001 | 0.002 | 0.004 | 0.004 |
| Standard 1 | 0.025 | 0.005 | 0.003 | 0.005 | 0.006 | |
| Standard 2 | 0.125 | 0.012 | 0.014 | 0.014 | 0.008 | |
| Standard 3 | 0.250 | 0.018 | 0.019 | 0.017 | 0.018 | |
| Standard 4 | 0.500 | 0.033 | 0.036 | 0.032 | 0.032 | |
| Standard 5 | 0.750 | 0.053 | 0.057 | 0.054 | 0.049 | |
| Standard 6 | 1.000 | 0.068 | 0.070 | 0.068 | 0.064 | |
| Standard 7 | 1.500 | 0.109 | 0.114 | 0.112 | 0.102 | |

*Figure 3: Enzyme quantitation methods used by IG.*
*A) SDS-polyacrylamide gel electrophoresis. Ladder in 1st lane, 2 µg BSA (~67 kDa) as a standard in lanes 1-3, and IG enzymes (~40 kDa) in lanes 4-6. The yellow boxes are the areas evaluated by densitometry. The integrated band intensities of IG enzymes are compared with integrated band intensities from BSA to assay concentration.*
*B) UV spectrum of a clean IG enzyme with protein peaks at 230 nm and at 280 nm. An extinction coefficient at 280 nm is typically used to quantify protein using these spectra with buffer subtraction at 330 nm.*
*C) BCA standard curve for BSA. The curve is used to calculate an IG enzyme concentration using BSA as the standard.*

Intact Genomics®

Intact Genomics owns the following registered trademarks granted by the United States Patent and Trademark Office (USPTO): Intact Genomics®, IG®, ig®, igTherapeutics®, FastAmp®, i7®, DirectPlate®.

All technology protocols discussed within this manual are assumed proprietary to Intact Genomics. This Product may be covered by pending or issued patents or may have certain limitations. Please contact us for more information. Purchase of this material conveys to buyer the non-transferable right to use the material purchased in research conducted by buyer, whether for teaching, non-commercial or commercial research purposes. Buyer may not sell or otherwise transfer these materials, its components, or unmodified descendants to a third party.

## Product Use Limitation and Disclaimers

This product is intended for research purposes only, not for therapeutic or diagnostic purposes in humans or animals. This product contains chemicals which may be harmful if misused or making direct human contact.

Intact Genomics is dedicated to practicing and maintaining science and technology ethics. Buyer agrees to use the purchased materials in full compliance with applicable law and regulations.

## Technical Support & Customer Services

Intact Genomics (IG®) is dedicated to customer satisfaction regarding the use of our products for your research needs. Each new lot of our products is thoroughly tested to ensure it meets the high quality standards and provide excellent results. We appreciate your business and your feedback regarding the performance of our products in your applications. Please follow the instructions carefully and contact us if additional assistance is needed.

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

**Intact Genomics, Inc.**
11840 Westline Industrial Drive, Suite 120,
St. Louis, MO. 63146, USA

**Phone:** (314) 942-3655 | **Toll-free :** 855-835-7172 | **Fax:** (314) 942-3656
**Email:** sales@intactgenomics.com | ig@intactgenomics.com
**Website:** www.intactgenomics.com

© 2024 Intact Genomics, Inc
All Rights Reserved