ig® Recombinase Polymerase Amplification (RPA) Kit

Manual

Catalog #	3526	3530
Package Size	25 Reactions	100 Reactions

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

Intact Genomics, Inc.

intactgenomics.com

Intact Genomics®

ig® RPA Kit Manual

Table of Contents

Description:

ig® Recombinase polymerase amplification (RPA) kit amplifies DNA at a single and constant temperature (37-42°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. 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). The IG® RPA Kit from Intact Genomics provides a mixture of the necessary enzymes to simplify and quicken the setup for your RPA reaction.

Kit Includes:

- Enzyme Mixture
- 2x RPA Reaction Master Mix
- 280 mM Magnesium Acetate (MgOAc)

Storage Temperature: -20°C

RPA Reaction Protocol:

1. Due to the viscosity of the 2X RPA Reaction Master Mix, it is recommended to, first, mix the DNA template, primers, and enzyme mixture on ice. Then, add the 2x RPA Reaction Master Mix.

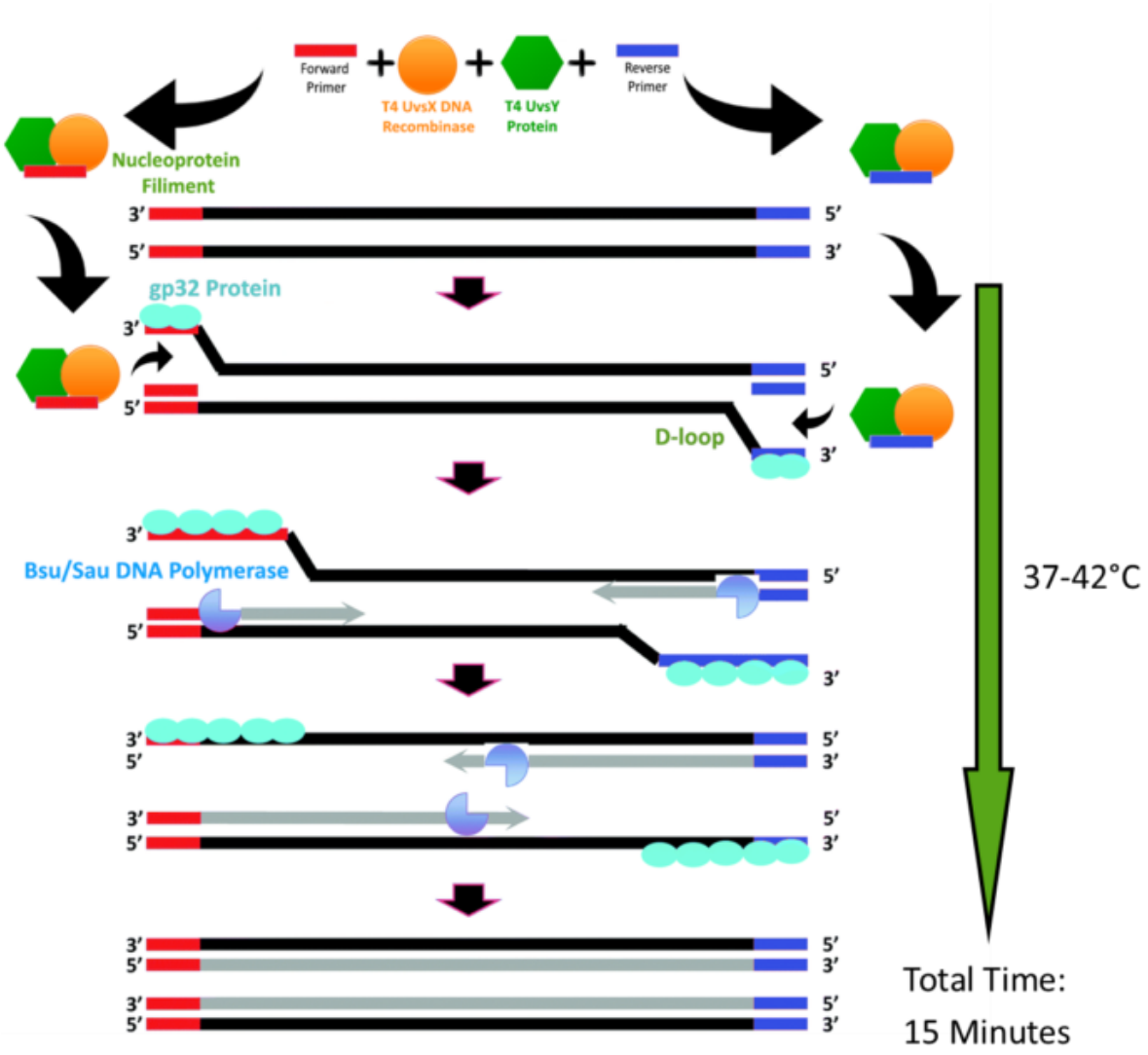
Component	Volume
DNA Template (10pg-100ng)	1.0 µl
Primer F (10 µM)	1.0 µl
Primer R (10 µM)	1.0 µl
Enzyme Mixture	6.0 µl
2x RPA Reaction Master Mix	10.0 µl

2. To initiate the reaction, add 1 µl of MgOAc, mix thoroughly, and pulse spin the reaction tube.

3. Incubate the tube at 38°C for 15-60 minutes. The reaction may also be performed at ambient temperature. However, we recommend increasing the incubation time to a minimum of 30 minutes.

4. Purify the DNA via ethanol precipitation (or the user's preferred method). Analyze the DNA by gel electrophoresis on a 1% agarose gel (or up to 2% agarose gel).

Schematic of Recombinase Polymerase Amplification:



Applications and Benefits:

- Highly selective and sensitive isothermal amplification technique.
- Alternative to PCR. No thermocycler needed.
- Speed and sensitivity. Excellent for rapid molecular and agricultural tests.
- No DNA pretreatment required.
- Flexible endpoint detection compatibility, (e.g. lateral flow, real-time fluorescence).
- All enzymes thoroughly tested for activity and purity.

Quality Control:

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

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. 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

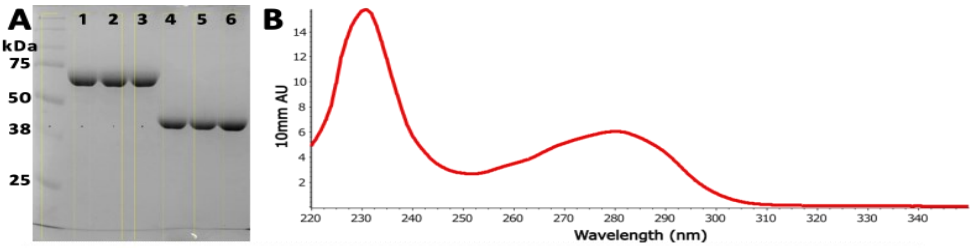
- Order online within the USA. Place orders on www.intactgenomics.com using our secure Shopping Cart.
- Order by email, phone, or fax.
  Email: sales@intactgenomics.com
  Phone: (314) 942-3655 | **Toll-free :** 855-835-7172 | **Fax:** (314) 942-3656
- Order from our distributors.

Enzyme Concentration:

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 1).

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.



C

BCA

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	

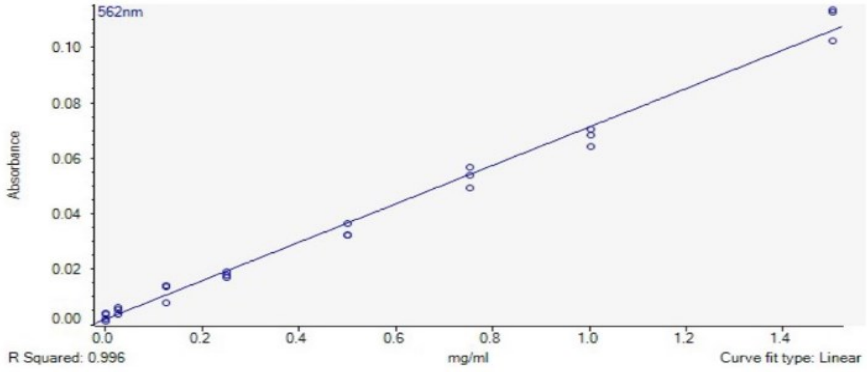


Figure 1: Enzyme quantitation methods used by IG. A) SDS-polyacrylamide gel electrophoresis. Ladder in 1st lane, 2 µg BSA (~67 kDa) as a standard in lanes 1-3, and IG enzymes (~40 kDa) in lanes 4-6. The yellow boxes are the areas evaluated by densitometry. The integrated band intensities of IG enzymes 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.

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