Intact Genomics®

Recombinase Polymerase Amplification Enhancer

Catalog #:	Package Size	Concentration
3522	50 µl (100 µg)	2 µg/µl
3525	250 µl (500 µg)	2 µg/µl

Description

Recombinase Polymerase Amplification (RPA) is an isothermal DNA amplification process that relies on the biological properties of three enzymes: a recombinase, a single-stranded (ss) DNA-binding protein (SSB) and a strand-displacing polymerase. T4 UvsX recombinase mediates DNA strand exchange between homologous chromosomes. The protein forms a right-handed nucleoprotein complex on single ssDNA called the presynaptic filament that can search for homology in duplex DNA and pair the recombining DNA molecules to form a DNA joint molecule (D-loop). This process is aided by ssDNA binding proteins and recombination mediators. The filament is then elongated by DNA polymerase with the newly synthesized strand displacing the old strand. The newly synthesized DNA can then act as a template for the next cycle to achieve exponential amplification of dsDNA at a constant temperature (25-42 ºC) (**1**). Although RPA is being used to detect human pathogens including bacteria, viruses, fungi, and parasites, it still has several limitations, such as: i) the sensitivity of the currently available TwistAmp® RPA kit (**2**) relative to PCR is less and dependent upon the source of template DNA used (**3**). ii) it can only amplify a short fragment of DNA which prevents its downstream applications such as large DNA cloning and gene fusion technology.

By screening different recombination proteins, Intact Genomics team has identified a novel RPA enhancer (**Fig. 2**). This RPA enhancer in combination with the recombinase (UvsX), recombinase-loading factors (Gp32, UvsY), specific strand-displacing polymerases, crowding agents (PEG) and robust ATP regeneration systems can significantly enhance the speed and yield of the currently available TwistAmp® basic RPA reaction (**Fig. 1**).

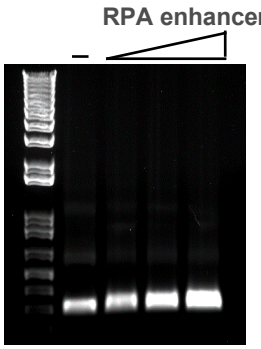

Fig. 1. Effect of increasing concentrations of RPA enhancer (50,100 and150 nM)

Protein Purity

The physical purity of this RPA Enhancer is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (Fig. 2).

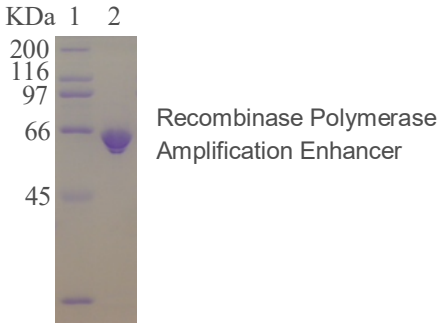

Fig. 2: Lane 1, Protein marker and lane 2, RPA Enhancer

Product Includes

- RPA Enhancer
- 10X RPA Buffer

RPA reaction buffer composition

50 mM Tris-HCl
50 mM KCl
5 mM MgCl2
0.3 mM MnCl2
0.1 mg/ml BSA
pH 7.6 @ 25ºC

Storage Buffer

50 mM Tris-HCl
50 mM KCl
1 mM DTT
0.1 mM EDTA
50% Glycerol
pH 7.5 @ 25ºC

Storage Temperature

-20ºC

Quality Control assays

Recombinase Polymerase Amplification Enhancer is free from detectable nuclease activities.

References

1. Piepenburg O, Williams CH, Stemple DL, and Armes NA. DNA detection using recombination proteins. PLoS Biol 2006; 4: e204.

2. TwistAmp® basic RPA kit (www.twistdx.co.uk).

3. Londoño MA, Harmon CL, Polston JE. Evaluation of recombinase polymerase amplification for detection of begomoviruses by plant diagnostic clinics. Virol J 2016; 13:48.

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