Innovative FastAmp® Viral and Cell Lysis Solution Enables Simple RPA-based PoC Diagnostics and Nucleic Acids Testing of Viral Pathogens

David Brent Halling, Peter Korajczyk, , Lukas Shelp, Jennifer Gormley, Rosa Ye, Chengcang C. Wu

Intact Genomics Inc., St Louis, Missouri, USA.

Contact information: Intact Genomics, Inc. | Phone: (314) 942-3655 | Fax: (314) 942-3656 | Email: cwu@intactgenomics.com | Website: www.intactgenomics.com

Abstract

Intact Genomics has developed FastAmp® Viral and Cell Lysit Solution (former name: FastAmp® Viral and Cell Solution for COVID-19 Testing) that can be utilized for saliva/swab samples resulting in viral/cell lysis and virus-inactivation at ambient temperature, significantly simplifying a PoC detection. The lysed viral DNA or RNA is ready for Recombinase Polymerase Amplification (RPA) reactions without the need for a DNA/RNA extraction step and it enhances the reactions. We have also developed and provided superior individual enzymes/proteins and/or custom-formulations of RPA and CRISPR reagents for all types of simple RPA-based PoC diagnostics and nucleic acids testing of viral pathogens. This FastAmp® solution provides an effective end-to-end solution to streamline specimen collection, DNA or RNA preparation processes including sample collection, transport, maintenance, viral/cell lysis and inactivation at room temperature, as well as viral RNA stabilization and the viral PoC detection.

Innovative FastAmp® viral and cell lysis solution

Intact Nuclease inactivation with FastAmp® solution

A concentrated (5×) version of FastAmp® viral and cell solution for Covid-19 testing was purchased directly from Intact Genomics. This solution, also referred to as FastAmp® lysis reagent or buffer, was used at a final concentration of 1×. For nuclease inactivation experiments, 10% healthy nasal fluid in UTM or pooled saliva samples (Lee BioSolutions) were mixed with 1× FastAmp® viral and cell solution and either 1%, 2% or 5% (v/v) murine RNase inhibitor (IG).

Viral inactivation with Intact Genomics FastAmp® solution

An isolate of SARS-CoV-2 (2019-nCoV/USA-WA1-A12/2020) was obtained from the CDC. At the Integrated Research Facility (IRF) – Frederick, the virus was passaged by inoculating grivet kidney epithelial Vero cells (ATCC CCL-81) at a multiplicity of infection of 0.01 under high containment (BSL-3) conditions. Infected cells were incubated for 48 or 72 h in Dulbecco's modified Eagle medium with 4.5 g/L D-glucose, L-glutamine and 110 mg/L sodium pyruvate (DMEM, Gibco) containing 2% heat-inactivated fetal bovine serum (SAFC Biosciences) in a humidified atmosphere at 37 °C with 5% CO2. The resulting viral seedstock was collected and quantified by plaque assay using Vero E6 cells (ATCC CRL-1586) with a 2.5% Avicel overlay and stained after 48 h with a 0.2% crystal violet stain.

For testing the viral inactivation capacity of the FastAmp® solution, the viral stock (10^7 PFU ml−1) was mixed with 1× lysis buffer supplemented with 5% (v/v) murine RNase inhibitor and incubated at ambient temperature for either 5 min, 20 min or 20 min followed by 10 min at 65°C. A no-treatment (PBS) control was also included. Treated viral stocks were then cleaned and quantified by plaque assay using Vero E6 cells (ATCC CRL-1586) with a 2.5% Avicel overlay and stained after 48 h with a 0.2% crystal violet stain. The results show infection not detected, zero plaque forming units (PFUs) in the FastAmp® solution at room temperature after 5 minutes (**Figure 2**).

Data Adapted from Arizti-Sanz, Bradley, Zhang, et al. medRxiv preprint doi:

https://doi.org/10.1101/2021.11.01.21265764, Improving the accessibility of SHINE assays.

For SARS-CoV-2 diagnostic testing to occur in virtually any location, improvements were needed to increase user-friendliness and facilitate test distribution. Use of an equipment-free and ambient-temperature sample lysis method with the FastAmp® solution further increases the user-friendliness of the assay. SHINEv.2 involves as few steps from the user as antigen-capture tests while providing a 50-fold boost in sensitivity. Importantly, SHINEv.2 demonstrates perfect (100%) concordance with RT-qPCR, the gold standard for SARS-CoV-2 diagnosis, in samples with RNA levels above our analytical LoD of 200 cp µl−1.

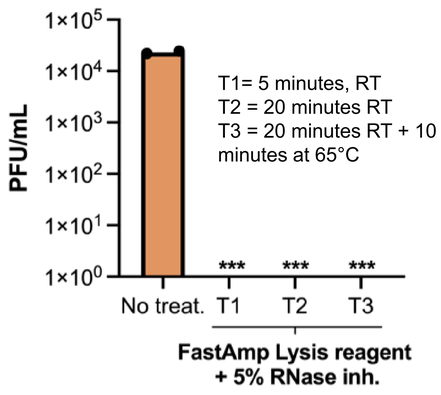


Figure 1. RNase inactivation in saliva by FastAmp® solution. RNase activity in pooled saliva samples untreated or treated with FastAmp Lysis solution supplemented with 5% RNase inhibitor or treated with HUDSON (a heat- and chemical- treatment). Activity measured using RNaseAlert at room temperature for 30 minutes. Centre = mean and error bars = s.d. for 3 technical replicates. Adapted from Qian, J., Boswell, S.A., Chidley, C. et al. An enhanced isothermal amplification assay for viral detection. Nat Commun 11, 5920 (2020). https://doi.org/10.1038/s41467-020-19258-y and Arizti-Sanz, J., Bradley, A., Zhang, Y.B. et al. Simplified Cas13-based assays for the fast identification of SARS-CoV-2 and its variants. Nat. Biomed. Eng 6, 932–943 (2022). https://doi.org/10.1038/s41551-022-00889-z.

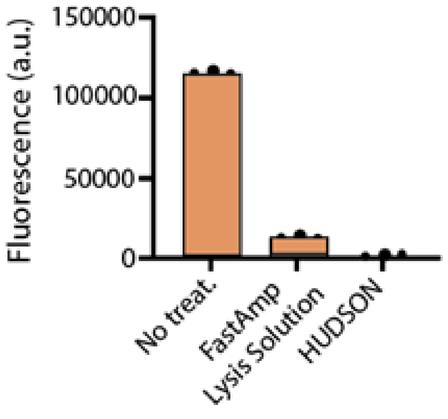


Figure 2. FastAmp® Solution inactivates SARS-CoV-2 viruses by plaque assays. SARS-CoV-2 seedstock titer without treatment or after being incubated with lysis reagent (+5% RNase inhibitor) at room temperature (RT) for 5 minutes (T1), 20 minutes (T2) or 20 minutes plus 10 minutes at 65°C (T3). ***, infection not detected; PFU, plaque forming units. Adapted from Arizti-Sanz, Bradley, Zhang, et al. medRxiv preprint 2021 doi: https://doi.org/10.1101/2021.11.01.21265764

FastAmp® Solution Key Features & Benefits

- No DNA or RNA extraction is needed.
- Safe for sample transport and maintenance.
- Speeds up testing processes.
- Compatible with various types of tissues including plants.
- Compatible with different detection technologies.
- Low toxicity to humans/environment.
- Results approved and published in top peer-reviewed journals.

FastAmp® Solution & Related Products

Product Name	Cat #	Pkg Size	Conc.
Exonuclease III	3412	10000 units	100 units/µl
	3415	25000 units	
Endonuclease IV (Nfo)	3422	2000 units	10 units/µl
	3425	5000 units	
RNase Inhibitor, Murine	3714	20,000 Units	
igScript™ Reverse Transcriptase	3344	50,000 units	200 u/µl
FastAmp® Viral and Cell Lysis Solution	4630	12.5 ml, 25 rxns	
	4631	50 ml, 100 rxns	
	4633	250 ml, 500 rxns	
	4636	10 ml, 5x, 100 rxns	

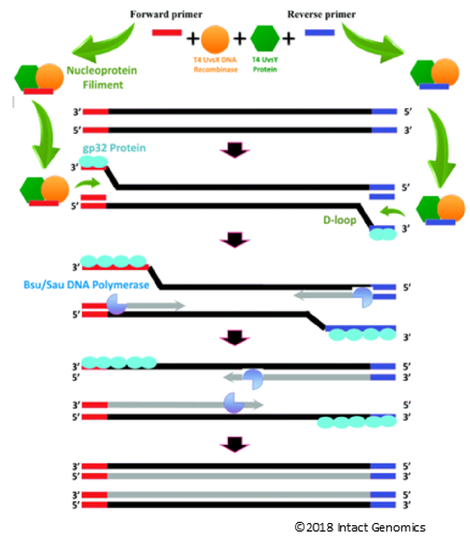
Superior individual enzymes/proteins and/or custom-formulations of Recombinase Polymerase Amplification (RPA)

Intact Genomics (IG) is a leading provider of Recombinase Polymerase Amplification (RPA) optimization products globally. RPA is a novel isothermal technology (Figure 3) that has been widely used for the molecular diagnosis of various infectious and other diseases in human, animals and plants.

Recombinase Polymerase Amplification (RPA) is an excellent candidate for developing rapid testing methods. However, glycerol is not compatible in all our customers' applications for RPA. Removing glycerol from an enzyme product takes time and it can be difficult. To satisfy customers' needs, Intact Genomics provides high-quality Glycerol-Free RPA enzymes, allowing customers to develop their own unique rapid testing tools for human health, agricultural applications, next-generation sequencing applications, and more.

We have also developed and provided superior individual enzymes/proteins and/or custom-formulations of RPA and CRISPR reagents for all types of simple RPA-based PoC diagnostics and nucleic acids testing of viral pathogens.

A Schematic of Recombinase Polymerase Amplification (RPA)



B

Technique	Typical Incubation Temp (°C)	Incubation
RPA	**Simple or Equipment-FREE** **25**-42°C	10-30 mins
PCR	**Thermocycler** 94°C –55°C –72°C	45-180 mins

Figure 3. The diagram A shows how RPA reaction works; B shows major difference RPA vs PCR.

RPA Applications & Benefits

- **Alternative to PCR.** Highly selective and sensitive isothermal DNA/RNA amplification
- **No Thermocycler or other heavy equipment needed.**
- **Fast Reaction & Cost-Effectiveness.**

Excellent for rapid point-of-care and on-site testing

- **Convenience & Simplicity.**
- **High-Quality & ISO Certified.**

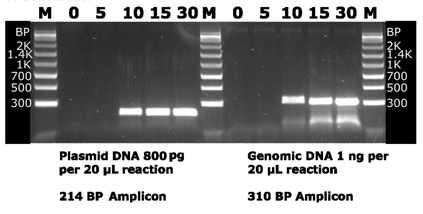
Intact Genomics uses proprietary methods to guarantee consistency and reliability.

- **RPA Enzyme Customization, Glycerol-free and Lyophilized Formulations Available.** We can meet your specific needs.

ig® RPA Kit v2

RPA Reaction Time (min)

M-DNA ladder



RPA-Related Enzymes

Product Name	Cat #	Pkg Size	Conc.
T4 UvsX DNA Recombinase	3562	100 µg	5µg/µl
	3565	500 µg	
	3567	1000 µg	
T4 UvsY Protein	3572	100 µg	2µg/µl
	3575	500 µg	
	3577	1000 µg	
T4 gp32 Protein	3511	100 units	5µg/µl
	3512	200 units	
	3515	500 units	
	3516	500 units	10 µg/µl
***Bsu* DNA Polymerase, Large Fragment**	3582	200 units	5units/µl
	3585	1000 units	
	3587	2000 units	
Sau DNA Polymerase, Large Fragment	3592	200 units	5units/µl
	3595	1000 units	

Glycerol-Free & Lyophilized RPA Enzymes

Product Name	Cat #	Pkg Size	Conc.
Glycerol-Free T4 UvsX DNA Recombinase	3562GF	100 µg	5µg/µl
	3565GF	500 µg	
	3567GF	1000 µg	
Lyophilized T4 UvsX DNA Recombinase	3562Lyo	100 µg	
	3565Lyo	500 µg	
	3567Lyo	1000 µg	
Glycerol-Free T4 UvsY Protein	3572GF	100 µg	2µg/µl
	3575GF	500 µg	
	3577GF	1000 µg	
Lyophilized T4 UvsY Protein	3572Lyo	100 µg	
	3575Lyo	500 µg	
	3577Lyo	1000 µg	
Glycerol-Free T4 gp32 Protein	3513GF	250 µg	10µg/µl
	3516GF	500 µg	
	3519GF	1000 µg	
Lyophilized T4 gp32 Protein	3513Lyo	250 µg	
	3516Lyo	500 µg	
	3519Lyo	1000 µg	
Glycerol-Free Bsu DNA Polymerase	3582GF	200 units	5 units/µl
	3585GF	1000 units	
Lyophilized Bsu DNA Polymerase	3582Lyo	200 units	
	3585Lyo	1000 units	

Intact Genomics' product quality management system is ISO 13485:2016 certified.

10 Minute DNA amplification Results!

Figure 4. ig® RPA v2 has been tested to various plasmid and genomic DNA targets to give results in just 10 minutes at 37ºC.

RPA reactions are stopped by moving samples to ice and immediately adding DNA dye with a 1% SDS solution. RPA also has robust results at controlled room temperature, 25ºC by extending the time to 20-45 minutes.

Conclusion

Intact Genomics' FastAmp® viral and cell lysis solution provides an effective end-to-end solution to streamline specimen collection, DNA or RNA preparation processes including sample collection, transport, maintenance, viral/cell lysis and inactivation at room temperature, as well as viral RNA stabilization and the viral PoC detection. Combining FastAmp® solution, RPA and CRISPR reagents for all types of simple RPA-based PoC diagnostics and nucleic acids testing of viral pathogens.

References

1. Qian, J., Boswell, S.A., Chidley, C. et al. An enhanced isothermal amplification assay for viral detection. Nat Commun 11, 5920 (2020). https://doi.org/10.1038/s41467-020-19258-y
2. Arizti-Sanz, Bradley, Zhang, et al. medRxiv preprint 2021 doi: https://doi.org/10.1101/2021.11.01.21265764
3. Arizti-Sanz, J., Bradley, A., Zhang, Y.B. et al. Simplified Cas13-based assays for the fast identification of SARS-CoV-2 and its variants. Nat. Biomed. Eng 6, 932–943 (2022). https://doi.org/10.1038/s41551-022-00889-z.
4. https://intactgenomics.com/product/dna-rna-and-cell-solution/.

Acknowledgments

We thank BEI Resources, a National Institute of Allergy and Infectious Diseases (NIH/NIAID) supported program managed by ATCC for providing the viral materials and samples.

Intact Genomics® Experts in Genomic Discovery

National Institute of Allergy and Infectious Diseases