

Saliva-Prep2 for High Throughput DNA Purification

Isohelix manufactures a well-established range of buccal and saliva DNA collection and isolation products, including the GeneFiX™ Saliva Collection device, which uses a patented funnel design for fast and simple collection of 1ml or 2ml saliva into a stabilization buffer, which has been shown to stabilize genomic DNA for at least 5 years at room temperature.

The method of DNA isolation is crucial to the yield and quality of the sample, especially when using sensitive downstream analysis methods.

Laboratories are processing larger numbers of samples; however, many High Throughput methods require investment in high cost machinery for automated isolation.

Isohelix has adapted the Saliva-Prep2 kit, a simple lysis and precipitation kit, for use with 1.2ml or 2.0ml 96 - Deep Well Plates. This gives a manual method for isolating 96 samples per plate, requiring only low-cost, widely available benchtop laboratory equipment. In this study the kit was used to purify 26 samples according to the manufacturer's instructions.

Results:

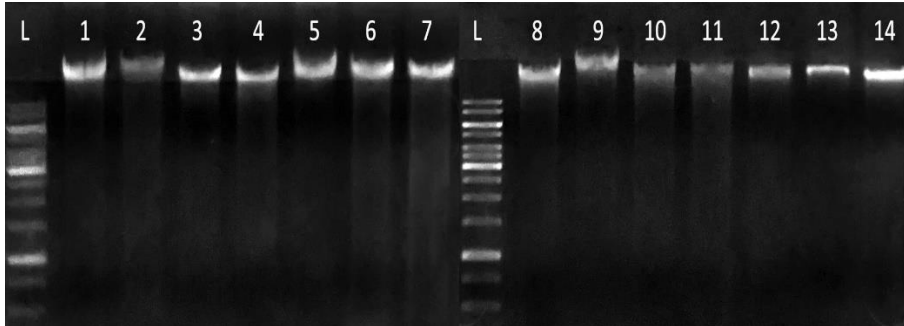
Yield was measured by Qubit and Nanodrop, purity was measured by A260/280 and A260/230 ratios on Nanodrop, and selected samples were run on 1.0% Agarose gel.

Sample Concentration & Purity (By Nanodrop):

| Sample | Nanodrop ng/μL | A260/280 | Sample | Nanodrop ng/μL | A260/280 |
|-------------|-------------------|----------|--------|-------------------|-------------|
| 1 | 166.35 | 1.82 | 14 | 177.49 | 1.81 |
| 2 | 333.02 | 1.83 | 15 | 62.50 | 1.83 |
| 3 | 58.13 | 1.80 | 16 | 49.54 | 1.73 |
| 4 | 160.29 | 1.80 | 17 | 64.72 | 1.83 |
| 5 | 90.17 | 1.76 | 18 | 58.41 | 1.76 |
| 6 | 50.32 | 1.71 | 19 | 29.31 | 1.80 |
| 7 | 82.33 | 1.79 | 20 | 83.88 | 1.76 |
| 8 | 109.2 | 1.82 | 21 | 164.35 | 1.76 |
| 9 | 140.44 | 1.79 | 22 | 48.03 | 1.80 |
| 10 | 77.11 | 1.78 | 23 | 127.25 | 1.76 |
| 11 | 51.93 | 1.71 | 24 | 64.67 | 1.80 |
| 12 | 94.99 | 1.85 | 25 | 206.23 | 1.79 |
| 13 | 84.56 | 1.81 | 26 | 193.61 | 1.80 |
| Mean | | | | 108.80 | 1.79 |

- **Sample concentrations show high yield across all samples, and absorbance ratios over 1.7 indicate high purity.**

1.0% Agarose Gel of Isolated HT Samples:



- Agarose gel samples show large clear bands of intact, high molecular weight genomic DNA.
- There is no evidence of shearing or RNA contamination.

Comparison between Manual and Deepwell plate (DWP) Methods

For a selection of samples, an equal volume was also isolated using the manual protocol given by the manufacturer's instructions, to give a direct comparison between the two methods.

| | DWP | Manual | DWP | Manual |
|--------|----------------------|--------|----------|--------|
| Sample | Nanodrop ng/ μ L | | A260/280 | |
| 1 | 166.35 | 147.40 | 1.82 | 1.86 |
| 7 | 58.13 | 50.87 | 1.80 | 1.96 |
| 13 | 50.32 | 78.93 | 1.71 | 1.81 |
| 20 | 109.20 | 139.11 | 1.82 | 1.87 |

- Good DNA Yields and Purity for use in NGS and other sensitive applications.
- Considerable time saving when compared to standard manual methods.
- Uses commonly available Laboratory Equipment.

Conclusions

- Ideally suited for laboratories with a high sample throughput requirement, where automated systems are unavailable.
- Manual and High Throughput methods use same reagents allowing processing flexibility depending on sample numbers.
- Samples isolated using the High Throughput protocol show high yields in the expected range for GeneFiX™ Saliva collectors.
- Sample purity from the High throughput protocol is excellent and matches that seen from the manual method.
- The protocol provides additional flexibility with regards to processing varying volumes of samples in a High Throughput environment.
- All reagents and plates used for the protocol are supplied, with no additional solvents or chemicals required.