Chromatrap

V 1.0

Chromatrap® HT DNA Purify and Concentrate

High throughput plates for the efficient purification and concentration of DNA

Protocol v1.0

Catalogue no. 500240

ADVANCEMENTS IN EPIGENETICS

HT DNA PURIFY AND CONCENTRATE

Contents

Introduction

Chromatrap® 96-well high throughput plates for ultra-pure DNA purification. Using proprietary filtration media that offer much higher loadings of active material, assay times are under 5 minutes. Buffers are optimised to remove any unwanted impurities while providing efficient DNA recovery from samples. The Chromatrap® HT DNA Purify and Concentrate kit is designed for the purification and concentration of samples from PCR mixtures, ChIP samples and restriction enzyme digestions.

The Chromatrap® HT DNA Purify and Concentrate kit can purify up to 10 µg DNA in small elution volumes (5-10 µl) providing a cleaner and more concentrated sample required for certain applications such as library preparation for DNA sequencing.

- *DNA samples ranging from 50 bp up to 23 kb can be purified with up to 98% recovery*
- *Up to 10 µg of DNA can be recovered efficiently and quickly using the Chromatrap® HT DNA Purify and Concentrate kit*

Kit Components and Storage

Kit Component	Quantity	Storage temperature
Chromatrap® HT DNA Purify and Concentrate plate	2	RT
96-well collection plate	2	RT
96-well elution plate	2	RT
DNA Binding Buffer	100 ml	RT
DNA Wash Buffer*	30 ml	RT
DNA Elution Buffer	2 ml	RT

The Chromatrap® HT DNA Purify and Concentrate kit (Cat no. 500240) and its contents can be stored for up to 12 months after the date of receipt without showing any reduction in performance and quality.

Chromatrap® products are intended for research purposes only.

Preparation of DNA Wash Buffer: Add 120 ml ethanol (96-100%) to the DNA Wash Buffer concentrate and note on the label that ethanol has been added.

WARNING: Some of the components of this product are irritants, refer to MSDS sheet for more information and follow safety guidelines of your research facility.

Additional materials required

- *Ethanol (96-100%)*
- *3M Sodium Acetate pH5.2*
- *Centrifuge with plate rotor*
- *Multi channel pipette*

For vacuum protocol only

- *Vacuum manifold (Porvair Sciences catalogue no. 228008)*
- *Vacuum pump*

Protocol

Using a centrifuge

1. Add 5 volumes DNA Binding Buffer to 1 volume of sample and mix.

DNA Binding Buffer contains an integrated pH indicator. DNA adsorption requires a pH of ≤7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5 the binding mixture will turn orange or violet meaning the pH of the sample exceeds the buffering capacity of the DNA Binding Buffer and DNA adsorption will be inefficient. In these cases, add 10 µl 3M sodium acetate, pH 5.2, to adjust the pH of the binding mixture. The colour of the mixture should turn yellow.

2. Place a Chromatrap® HT DNA Purify and Concentrate plate and position on to the 96-well collection plate provided.
3. Transfer each sample to a corresponding well on the Chromatrap® 96 HT DNA Purify and Concentrate plate.
4. Centrifuge at 3,000xg for 60 seconds at RT. Discard the flow through.
5. Add 700 µl DNA Wash Buffer to each well and centrifuge at 3,000xg for 60 seconds at RT. Discard the flow through.
6. Spin dry at 3,000xg for 30 seconds at RT to remove any remaining liquid from the plate.
7. Transfer the Chromatrap® HT DNA Purify and Concentrate plate on to a clean supplied 96-well DNA Elution plate.
8. To elute DNA, add 5-10 µl DNA Elution Buffer to the centre of the frit in each well and incubate for 1 minute. Centrifuge at 3,000xg for 60 seconds to collect the eluted DNA.

Using a vacuum manifold

1. Add 5 volumes DNA Binding Buffer to 1 volume of sample and mix.

DNA Binding Buffer contains an integrated pH indicator. DNA adsorption requires a pH of ≤7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5 the binding mixture will turn orange or violet meaning the pH of the sample exceeds the buffering capacity of the DNA Binding Buffer and DNA adsorption will be inefficient. In these cases, add 10 µl 3M sodium acetate, pH 5.2, to adjust the pH of the binding mixture. The colour of the mixture should turn yellow.

2. Place a Chromatrap® 96-well collection plate in the bottom of the vacuum manifold with enough spacers to ensure a seal is obtained when the Chromatrap® HT DNA Purify and Concentrate plate is positioned on top.
3. Transfer each sample to a corresponding well on the Chromatrap® HT DNA Purify and Concentrate plate.
4. Switch on the vacuum to pull the sample through the well (0.1-0.2 Bar).
5. Turn off the vacuum once all the sample has passed through to ensure equal vacuum is applied to all samples. Discard the flow through.
6. Add 700 µl DNA Wash Buffer to each well and switch on the vacuum to pull the sample through the well (0.1-0.2 Bar). Turn off the vacuum once all the sample has passed through and discard the flow through.
7. Switch on the vacuum for 5 minutes to ensure all liquid is removed from the membrane.
8. Place a clean 96-well elution plate in the bottom of the vacuum manifold with enough spacers to ensure a seal is obtained when the Chromatrap® HT DNA Purify and Concentrate plate is placed on top.
9. To elute DNA, add 5-10 µl DNA Elution Buffer to the centre of the membrane of each well and incubate for 1 minute. Switch on the vacuum for 1 minute to elute the sample (0.2-0.4bar).

Appendix

Quantitative DNA recovery following DNA purification

The Chromatrap® HT DNA Purify and Concentrate kit is capable of purifying a range of DNA concentrations 0.1 to 10 µg (Figure 1).

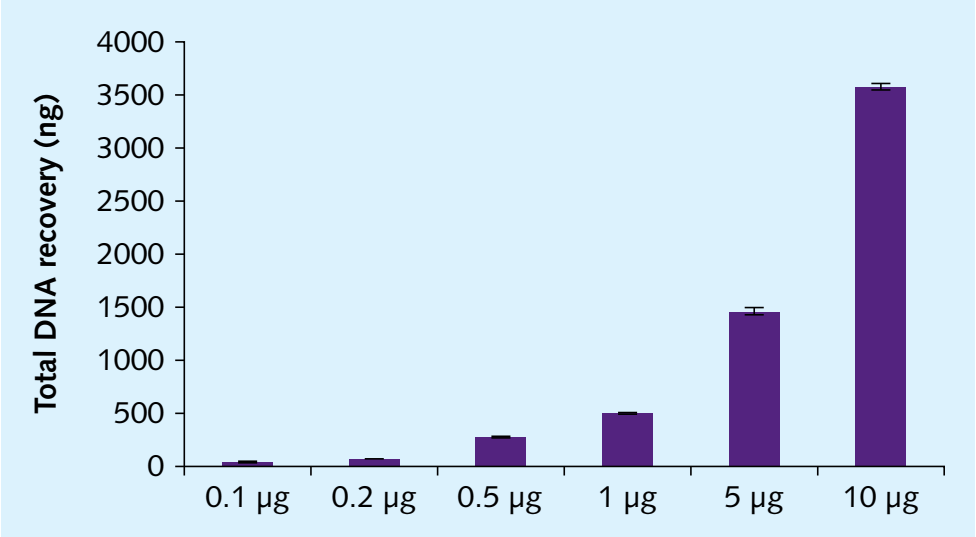

Figure 1. *Total DNA recovery (ng) of a range of DNA concentrations (0.1-10 µg) using the Chromatrap® HT DNA Purify and Concentrate kit, samples were eluted in 10 µl DNA Elution Buffer.*

Lower and upper size limits

DNA fragments ranging from 50 bp up to 23 kb in size can be purified using Chromatrap® HT DNA Purify and Concentrate kit. 10 µl of digested or undigested DNA was purified using the Chromatrap® HT DNA Purify and Concentrate kit and eluted in 5 or 10 µl DNA Elution Buffer. The purified sample was then run on a 2% agarose gel to determine the relative intensity of the purified sample compared to an unpurified ladder as a control. The unpurified ladder was diluted 1:5 and an equivalent volume (30 µl) added to the gel (figure 2). Staining of the gel indicates that the Chromatrap® HT DNA Purify and Concentrate kit is capable of purifying DNA fragment sizes from 50 bp to 23 kb.

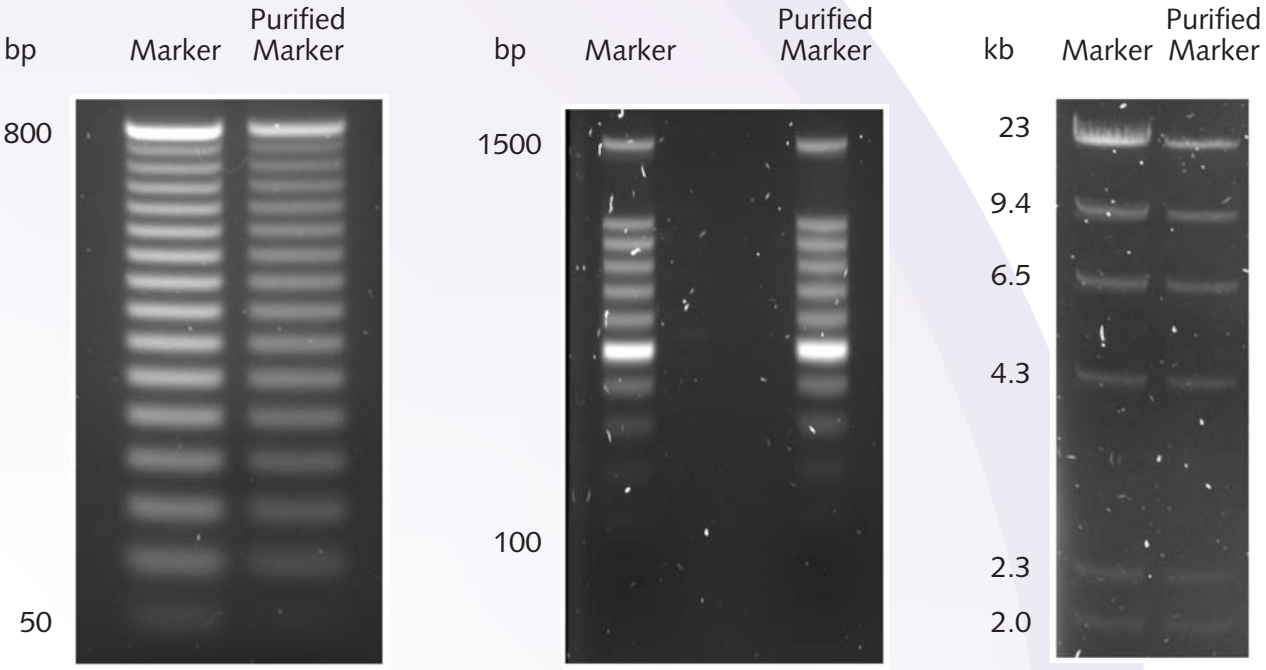

Figure 2. *2% agarose gels of unpurified and purified ladders.*

DNA size	Percent recovery
50 bp to 1 kb	50 – 80%
100 bp to 1.5 kb	60 – 100%
2 kb to 23 kb	70 – 90%

Table 1. *DNA percentage recovery from relative band intensities*

Sample type	ChIP samples, PCR samples, enzymatic reactions
DNA recovery	10 µl elution – up to 98%
Size limits	50 bp - 23 kb
Max binding capacity	10 µg
Elution volume	5-10 µl
Downstream applications	PCR, sequencing, microarray, ligation, cloning

Table 2. *Product specifications*

Troubleshooting Guide and FAQs

1. I have very little DNA recovery following DNA purification, why?

Ensure ethanol (96-100%) has been added to the DNA Wash Buffer. The ethanol helps the DNA to precipitate and bind to the frit. When the DNA Binding Buffer is added to your sample, check that the solution is 'yellow' and not violet/orange. DNA adsorption occurs at a pH ≤ 7.5, therefore the correct pH is paramount to ensure the DNA binds to the frit. In these cases, add 10 µl 3M sodium acetate, pH 5.2, to adjust the pH of the binding mixture. The colour of the mixture should turn yellow. Check that the DNA Elution Buffer is added to the centre of each well of the Chromatrap® HT DNA Purify and Concentrate plate to ensure that the DNA Elution Buffer completely covers the frit. This is particularly important when using small elution volumes (5-10 µl).

2. My purity ratio is low/below the limits which indicates the sample is not pure

This suggests the DNA Wash Buffer was not completely removed after the dry centrifugation step. An additional dry spin (3,000xg for 30 seconds at RT) should ensure the removal of any residual DNA Wash Buffer from your sample.

3. My sample floats out of the well when trying to load a gel

This indicates ethanol carryover. The DNA Wash Buffer may not have been completely removed after the dry centrifugation step. Try an additional dry spin (3,000xg for 30 seconds at RT) to ensure the removal of any residual DNA Wash Buffer from your sample. Heating the eluted samples at 56°C for 10 minutes before loading the gel will help to evaporate any residual ethanol.

4. Can I increase the elution volume?

This kit has been optimised for an elution volume of 5-10 µl. A 96 DNA Purification Kit (Cat no. 500220) for purification of up to 50 µg DNA in larger elution volumes is also available from Chromatrap®.

Other products available from Chromatrap®

ChIP products

Product	Quantity	Catalogue no.
Chromatrap® ChIP-seq Pro A	24	500189
Chromatrap® ChIP-seq Pro G	24	500190
Chromatrap® HT ChIP-seq Pro A	1 x 96	500214
Chromatrap® HT ChIP-seq Pro G	1 x 96	500215
Chromatrap® Enzymatic ChIP-seq Pro A	24	500191
Chromatrap® Enzymatic ChIP-seq Pro G	24	500192
Chromatrap® HT Enzymatic ChIP-seq Pro A	1 x 96	500216
Chromatrap® HT Enzymatic ChIP-seq Pro G	1 x 96	500217
Chromatrap® ChIP qPCR Pro A	24	500071
Chromatrap® ChIP qPCR Pro G	24	500117
Chromatrap® Premium ChIP qPCR Pro A	24	500115
Chromatrap® Premium ChIP qPCR Pro G	24	500116
Chromatrap® HT ChIP qPCR Pro A	1 x 96	500161
Chromatrap® HT ChIP qPCR Pro G	1 x 96	500163
Chromatrap® HT Enzymatic ChIP qPCR Pro A	1 x 96	500162
Chromatrap® HT Enzymatic ChIP qPCR Pro G	1 x 96	500164
Chromatrap® Enzymatic ChIP qPCR Pro A	24	500166
Chromatrap® Enzymatic ChIP qPCR Pro G	24	500168
Chromatrap® Premium Enzymatic ChIP qPCR Pro A	24	500167
Chromatrap® Premium Enzymatic ChIP qPCR Pro G	24	500169
Chromatrap® FFPE ChIP-seq Pro A	24	500235
Chromatrap® FFPE ChIP-seq Pro G	24	500236
Chromatrap® Native ChIP-seq Pro A	24	500237
Chromatrap® Native ChIP-seq Pro G	24	500238
Chromatrap® Sonication Shearing		500239
Chromatrap® Enzymatic Shearing		500165

DNA products

Product	Quantity	Catalogue no.
Chromatrap® DNA Purification	50	500218
Chromatrap® Gel Purification	50	500219
Chromatrap® HT DNA Purification	2 x 96	500220
Chromatrap® HT DNA Purify and Concentrate	2 x 96	500240
Chromatrap® DNA Extraction	50	500260
Chromatrap® HT DNA Extraction	2 x 96	500261
Chromatrap® Size Selection	50	500262
Chromatrap® HT Size Selection	2 x 96	500263

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