Chromatrap

V 1.1

Chromatrap® DNA Extraction

Chromatrap® spin columns for the efficient extraction and purification of total DNA from cells, tissues, whole blood and serum

Protocol v1.1

Catalogue no. 500260

ADVANCEMENTS IN EPIGENETICS

DNA EXTRACTION

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Introduction

The Chromatrap® DNA Extraction kit is a quick, easy and efficient way to perform total (genomic and mitochondrial) DNA extraction and purification. The Chromatrap® DNA Extraction kit can be used to effectively extract excellent quality DNA from cultured cells, tissues, whole blood and serum. The ultra pure DNA obtained with Chromatrap® solid state technology is suitable for a variety of downstream applications including PCR, MeDIP, Southern blotting, cloning and sequencing.

Highlights of protocol v1.1

- **DNA extraction from whole blood and serum**
- *Unique buffer chemistry allows efficient cell lysis*
- *No phenol or chloroform*
- *Simple extraction with easy centrifugation steps and minimal handling time*
- *Quick protocol – DNA is extracted in 30 minutes*
- *Excellent high quality and pure DNA obtained*

DNA Extraction protocol overview

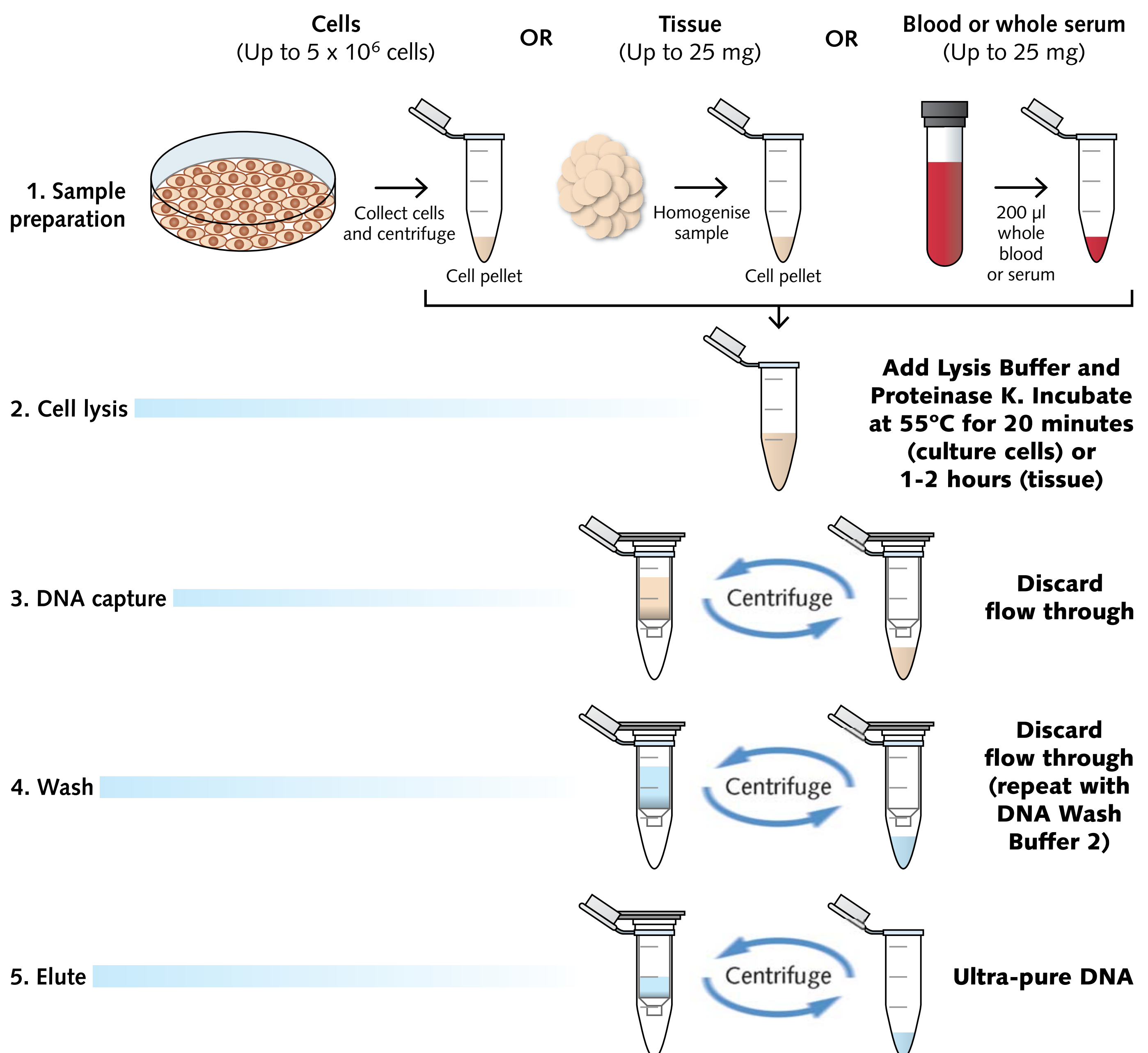

Figure 1: *Overview of the DNA extraction process*

Kit components and storage

Kit component	Quantity	Storage temperature
Chromatrap® DNA Extraction columns	50	RT
Proteinase K	520 µl	-20°C (shipped at RT)
DNA Extraction Lysis Buffer*	12 mls	RT
DNA Extraction Wash Buffer 1*	6 mls	RT
DNA Extraction Wash Buffer 2	6 mls	RT
DNA Extraction Elution Buffer	12 mls	RT

** Contains chaotropic agent*

The Chromatrap® DNA Extraction kit (Cat no. 500260) 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 1 and 2: DNA Wash Buffer 1 and 2 are supplied as concentrate. Add 24 mls ethanol (96-100%) to DNA Wash Buffer 1 and 2 and note on the labels 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

- Trypsin (if using adherent cells)
- RNase A
- PBS
- Waterbath
- Vortex
- Microcentrifuge
- 1.5 ml microcentrifuge tubes
- Ethanol (96-100%)

Protocol

The following section describes the method for total DNA extraction and purification using cultured cells (protocol A), tissue (protocol B) and whole blood or serum (protocol C).

Protocol A: For cultured cells

DNA can be extracted from up to 5 x 10^6 cells.

Step 1: Cell Preparation

This section describes cell preparation for both adherent (step 1a) and suspension (step 1b) cells.

Step 1a: For adherent cells

1. Culture up to 5 x 10^6 cells.
2. Detach the cells from the culture vessel. This can be done by trypsinisation or by cell scraping.
3. Add sufficient PBS to cover the culture vessel (RT).
4. Collect the cells by centrifugation at 200xg for 5 minutes at RT and remove supernatant.
5. Re-suspend cell pellet in 200 µl PBS and transfer each sample to a clean dry 1.5 ml centrifuge tube.

Step 1b: For suspension cells

1. Culture up to 5 x 10^6 cells.
2. Collect the cells by centrifugation at 200xg for 5 minutes at RT.
3. Re-suspend cell pellet in 200 µl PBS and transfer each sample to a clean dry 1.5 ml centrifuge tube.

Step 2: Cell Lysis

Note, some precipitates form in the DNA Extraction Lysis Buffer. Mix well before each use by vortexing.

1. Add 10 µl Proteinase K to each sample and mix well by pipetting.
2. Add 200 µl DNA Extraction Lysis Buffer to each sample (vortex DNA Extraction Lysis Buffer before use) and mix sample well by pulse vortexing for 30 seconds.
3. Incubate samples for 20 minutes at 55°C.
4. Following incubation, briefly centrifuge the samples to remove any liquid from the caps.

Step 3: DNA Capture

1. Add 200 µl ethanol (96-100%) to each sample and mix well by vortexing.
2. Briefly centrifuge the samples to remove any liquid from the caps.
3. Load each sample onto a corresponding Chromatrap® DNA Extraction column. Centrifuge at 10,000xg for 1 minute at RT. Discard the flowthrough.

Step 4: Wash

1. Add 500 µl DNA Extraction Wash Buffer 1 to each column and centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.
2. Add 500 µl DNA Extraction Wash Buffer 2 to each column and centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.
3. Spin dry at full speed for 1 minute at RT to remove any remaining liquid from each column. The original collection tubes should be discarded at this point and columns transferred into clean dry 1.5 ml micro centrifuge tubes.

Step 5: Elute

1. Add 200 µl DNA Extraction Elution Buffer or nuclease free water to each column, cap and incubate for 5 minutes at RT.
2. Centrifuge the columns at full speed for 1 minute at RT to collect the eluted DNA.

Protocol B: For tissue

For tissue, up to 25mg tissue can be extracted. Determine tissue weight by weighing each sample.

Step 1: Sample Preparation

1. Cut tissue into small samples using a sterile scalpel and transfer to a 1.5ml micro centrifuge tube.

Step 2: Cell Lysis

Note, some precipitates form in the DNA Extraction Lysis Buffer. Mix well before each use by vortexing.

1. Add 200 µl DNA Extraction Lysis Buffer to each sample (vortex DNA Extraction Lysis Buffer before use) and mix well by pulse vortexing for 30 seconds.
2. Add 10 µl Proteinase K to each sample and mix well by pipetting.
3. Incubate samples at 55°C for 1-2 hours or until the tissue has completely lysed and no traces of the tissue can be seen.
4. Following incubation, briefly centrifuge the samples to remove any liquid from the caps.

Step 3: DNA Capture

1. Add 200 µl ethanol (96-100%) to each sample and mix well by vortexing.
2. Briefly centrifuge the samples to remove any liquid from the caps.
3. Load each sample onto a corresponding Chromatrap® DNA Extraction column. Centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.

Step 4: Wash

1. Add 500 µl DNA Extraction Wash Buffer 1 to each column and centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.
2. Add 500 µl DNA Extraction Wash Buffer 2 to each column and centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.
3. Spin dry at full speed for 1 minute at RT to remove any remaining liquid from each column. The original collection tubes should be discarded at this point and columns transferred into clean dry 1.5 ml centrifuge tubes.

Step 5: Elute

1. Add 200 µl DNA Extraction Elution Buffer or nuclease free water to each column, cap and incubate for 5 minutes at RT.
2. Centrifuge the columns at full speed for 1 minute at RT to collect the eluted DNA.

Protocol C: For whole blood and serum

DNA can be extracted using 200 µl whole blood or serum.

Step 1: Cell Lysis

Note, some precipitates form in the DNA Extraction Lysis Buffer. Mix well before each use by vortexing.

1. Transfer 200 µl whole blood or serum to a clean dry 1.5ml centrifuge tube.
2. Add 10 µl Proteinase K to each sample and mix well by pipetting.
3. Add 200 µl DNA Extraction Lysis Buffer to each sample (vortex DNA Extraction Lysis Buffer before use) and mix sample well by pulse vortexing for 30 seconds.
4. Incubate samples for 20 minutes at 55°C.
5. Following incubation, briefly centrifuge the samples to remove any liquid from the caps.

Step 2: DNA Capture

1. Add 200 µl ethanol (96-100%) to each sample and mix well by vortexing.
2. Briefly centrifuge the samples to remove any liquid from the caps.
3. Load each sample onto a corresponding Chromatrap® DNA Extraction column. Centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.

Step 3: Wash

1. Add 500 µl DNA Extraction Wash Buffer 1 to each column and centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.
2. Add 500 µl DNA Extraction Wash Buffer 2 to each column and centrifuge at 10,000xg for 1 minute at RT. Discard the flow through.
3. Spin dry at full speed for 1 minute at RT to remove any remaining liquid from each column. The original collection tubes should be discarded at this point and columns transferred into clean dry 1.5 ml micro centrifuge tubes.

Step 5: Elute

1. Add 200 µl DNA Extraction Elution Buffer or nuclease free water to each column, cap and incubate for 5 minutes at RT.
2. Centrifuge the columns at full speed for 1 minute at RT to collect the eluted DNA.

Appendix

Checking DNA yield and purity

Following elution, the DNA yield can be quantified by measuring the absorbance at 260 nm using a spectrophotometer. The purity of the DNA can be determined by assessing the A260/A280 ratio. For pure DNA, the A260/A280 ratio is 1.8-2.2. If the A260/A280 ratio is higher or lower, this suggests there are contaminants in the samples (see troubleshooting guide).

The Chromatrap® DNA Extraction kit efficiently extracts and purifies total DNA from a wide range of cell numbers.

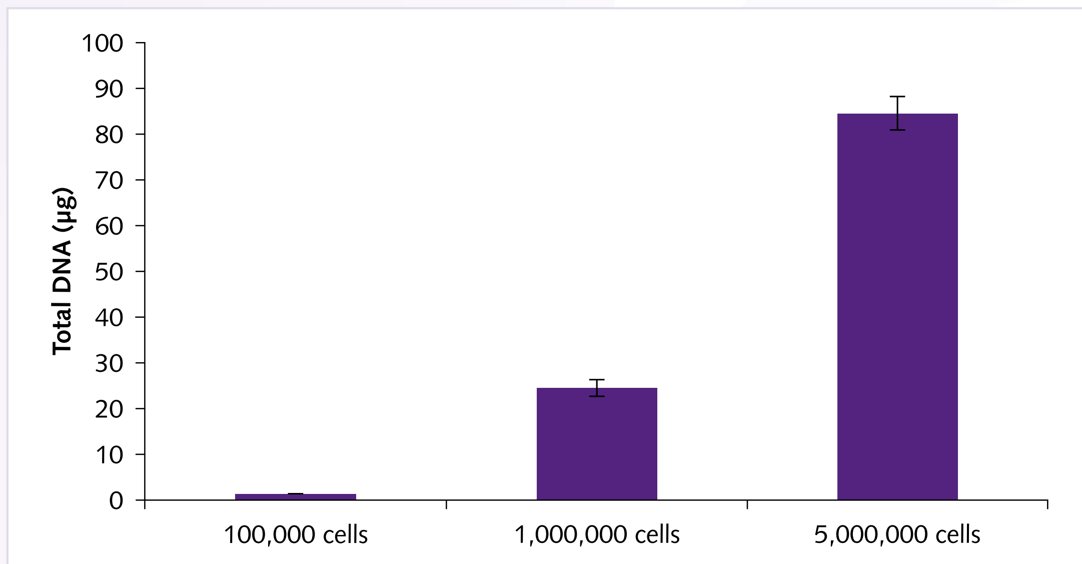

Figure 1. DNA was extracted from an endometrial cancer cell line using a range of cell numbers ($1x10^5$ to $5x10^6$) using the Chromatrap® DNA Extraction kit. All experiments were performed in triplicate.

The Chromatrap® DNA Extraction kit outperforms competitor extraction kits in both DNA yield and purity. Chromatrap® recovers the most DNA from the same cell number when compared to competitor kits with highest reproducibility.

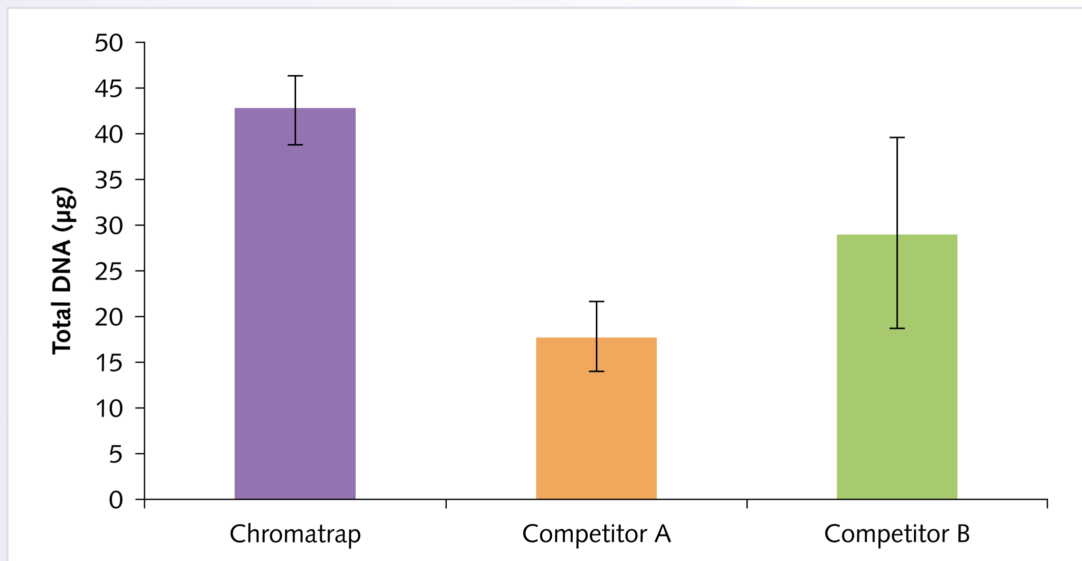

Figure 2. DNA was extracted from an endometrial cancer cell line using $2x10^6$ cells using the Chromatrap® DNA Extraction kit or competitor DNA Extraction kits. All experiments were performed in triplicate.

Troubleshooting Guide and FAQs

1. There are precipitates in the DNA Extraction Buffer

It is normal for precipitates to form in the DNA Extraction Buffer. Mix well before each use by vortexing.

2. How long should cells/tissue be lysed?

If using cell lines, cells should be efficiently lysed after incubating for 20 minutes at 55°C. If using tissue, efficient cell lysis will differ depending on tissue type. We recommend to incubate samples at 55°C for 1-2 hours or until the tissue has completely lysed and no traces of the tissue can be seen.

3. How do I ensure cells/tissue are completely lysed?

Ensure adequate DNA Extraction Lysis Buffer volume has been used for the number of cells being processed. Make sure samples are re-suspended thoroughly before incubating samples at 55°C. For tissue, lysis is complete when no traces of tissue are visible. If bits of tissue are still visible after 1-2 hours of incubation, add an extra 50 µl DNA Extraction Lysis Buffer, mix well and incubate until there are no traces of tissue. Cutting the tissue into small pieces will decrease lysis incubation time.

4. I forgot to add ethanol to my sample before loading onto the DNA Extraction Column

The addition of ethanol is important for DNA precipitation. Without the addition of ethanol, DNA capture will be less efficient, reducing DNA yield. Repeat DNA extraction with a new sample.

5. The sample will not pass through the DNA Extraction column/the column has blocked

This suggests the cells/tissue have not completely lysed causing the DNA Extraction column to block. Repeat the centrifugation step increasing the speed to full speed until the sample has passed through the DNA Extraction column.

6. I have forgotten to add ethanol to DNA Extraction Wash Buffers 1 and 2

It is important the correct volumes of ethanol (96-100%) have been added to DNA Extraction Wash Buffers 1 and 2 before use or purification will not work. If wash buffer concentrates have been used, repeat DNA extraction with a new sample. Prepare correct working concentrations of the wash buffers by diluting concentrate wash buffers 1:4 with 96-100% ethanol (e.g. 10 ml concentrate buffer and 40 ml 96-100% ethanol for 50 ml total volume).

7. My purity ratios (260/280) are low which indicates the sample is not pure

This suggests DNA Extraction Wash Buffer 2 was not completely removed after the dry centrifugation step. It is important to completely remove any ethanol from the sample as it may interfere with downstream processes. An additional dry spin (full speed for 1 minute at RT) should ensure the removal of any residual DNA Wash Buffer from your sample.

8. My sample floats out of the well when trying to load an agarose gel

This indicates ethanol carryover in the samples. DNA Extraction Wash Buffer 2 may not have been completely removed after the dry centrifugation step. Try an additional dry spin (full speed for 1 minute 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.

9. Can I reduce the elution volume?

This kit has been optimised for an elution volume of 200 µl. Elution volume can be reduced but may lower DNA yield.

10. Shall I elute my samples in Chromatrap DNA Extraction Elution Buffer or water?

Samples can be eluted in Chromatrap® DNA Extraction Elution Buffer or water depending on the downstream process. However we recommend using Chromatrap® DNA Extraction Elution Buffer for long-term storage to maintain DNA integrity as DNA is more susceptible to acid hydrolysis when stored in water.

11. Do I need to treat my samples with RNase A following DNA extraction?

If using a spectrophotometer to determine DNA concentration, samples contaminated with RNA can overestimate the concentration of DNA. To ensure your samples are free from RNA, we recommend performing a RNase A treatment. Following the elution step, add RNase A for 1 hour at 37°C to efficiently degrade RNA.

12. What cell types have been validated for use with this protocol?

This protocol has been optimised for both adherent and suspension cell lines, tissues, whole blood and serum. DNA extraction from different sources will need to be optimised by the user.

13. How many cells/how much tissue do I use for each DNA extraction?

For cultured cells, do not use more than 5 x 10^6 cells. For tissue, do not use more than 25 mg of tissue. The best way to determine tissue weight is by weighing each sample.

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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