VIA Extractor™ tissue disaggregator

GENTLE, EFFICIENT TISSUE DISAGGREGATION FOR OPTIMIZED CELL VIABILITY AND YIELD

The VIA Extractor™ tissue disaggregator* (Fig 1A) is a first-in-kind device for the disaggregation of human and animal solid tissue and tumor samples into viable, single cells. The VIA Extractor™ tissue disaggregator uses a mild processing approach for consistently high cell viability, yield, and preservation of cell integrity relative to the parent sample. The standardized, closed system provides a semi-automated process for use in high-throughput omics research (genomics, proteomics, metabolomics etc.) giving reliable results in single-cell sequencing and flow cytometry applications.

- Gentle: Optimized cell viability and yield from low impact disaggregation for optimal results in omics and single-cell sequencing applications.
- Standardized: Consistent process, output and yield reduce sample-to-sample variation.
- Semi-automated: Simpler process with fewer components and steps for tissue dissociation than traditional methods.
- Fast: Solid tissue to single cell suspension in as little as ten minutes.

Following disaggregation, cells can be preserved by controlled-rate freezing using VIA Freeze™ Uno controlled-rate freezer to maintain viability for further downstream applications.

The fresh tissue or tumor sample is processed within the Omics pouch, a barcoded, multi-compartment single-use bag. The sample is inserted into the chosen compartment using the Omics applicator. The sample bag is placed into the Omics clamp for extra stability and then the bag is heat sealed for further protection against contamination. A digestive enzyme solution is easily added to the Omics pouch via the ports using a syringe. The Omics pouch and clamp are carefully placed into the VIA Extractor™ tissue disaggregator (Fig 1B). The VIA Extractor™ tissue disaggregator is placed into the top of the VIA Freeze™ Uno controlled-rate freezer (Fig 1C) and the tissue disaggregation protocol is selected. The VIA Freeze™ Uno controlled-rate freezer offers flexibility by allowing the optimal speed, temperature and time settings to be selected depending on the sample type and size to maximize cell viability. The heating system of the VIA Freeze™ Uno controlled-rate freezer is used to control temperature. The gentle movement of the paddles in the VIA Extractor™ tissue disaggregator completes the mechanical disagreggation process resulting in a viable cell suspension, representative of the original sample.

Fig 1. (A) The VIA Extractor™ tissue disaggregator, (B) the Omics pouch placed into the VIA Extractor™ tissue disaggregator and held in place with the Omics clamp, and (C) the VIA Extractor™ tissue disaggregator placed into the top of the VIA Freeze™ Uno controlled-rate freezer.

The following data was generated to demonstrate the effectiveness of the VIA Extractor™ tissue disaggregator for disaggregation of biological tissues into a viable cell suspension that is suitable for use for a wide range of applications, including tumoroid growth, flow cytometry, and single cell sequencing and analysis. The VIA Extractor™ tissue disaggregator was used alongside the popular manual method to disaggregate a selection of commonly studied tissues according to standard protocols to determine relative performance in generating viable cell suspensions.

*For research use only (RUO). Not for diagnostic use.

Comparative performance of the VIA Extractor™ tissue disaggregator with manual tissue dissociation

Traditionally, solid biological tissues are dissociated into single cell suspension by dissection of the tissue into small pieces (2–4 mm) using scissors and scalpels, followed by long incubation periods in enzyme cocktails to release the cells. This technique is time consuming, labor intensive and less reproducible. Tissue dissociation was compared using three independent mouse organs, with half the material used for each technique. Following tissue dissociation, all cell suspensions were filtered through an appropriately sized cell strainer, pelleted by centrifugation at 300 xg and subject to red blood cell lysis using the Red Blood Cell Lysis Solution (Miltenyi Biotec). Following cell resuspensions, cells were counted using the NucleoCounter™ NC-200™ automated cell counter.

Mouse liver disaggregation

For manual disaggregation, mouse liver tissue (400–500 mg) was minced using scalpel and scissors, washed and incubated at 37°C on rotation with 0.1% Collagenase A for one hour. For comparison, mouse liver tissue (400–500 mg) was disaggregated by placing in the Omics pouch with 0.1% Collagenase A and running the VIA Extractor™ tissue disaggregator for 10 minutes at 200 rpm at 37°C. The residual tissue in the cell strainer following manual tissue disaggregation is indicative of the less efficient process, whereas a complete cell suspension is achieved with VIA Extractor™ tissue disaggregator in a fraction of the time (Fig 2A). The viability of cells is higher using the VIA Extractor™ tissue disaggregator as is the yield of viable cells (Figure 2B and 2C). The cell suspension as a result of VIA Extractor™ tissue disaggregator disaggregation compared to manual has a larger number of cells at the desired size and fewer small particles, suggesting a purer sample with reduced cell damage (Fig 2D).

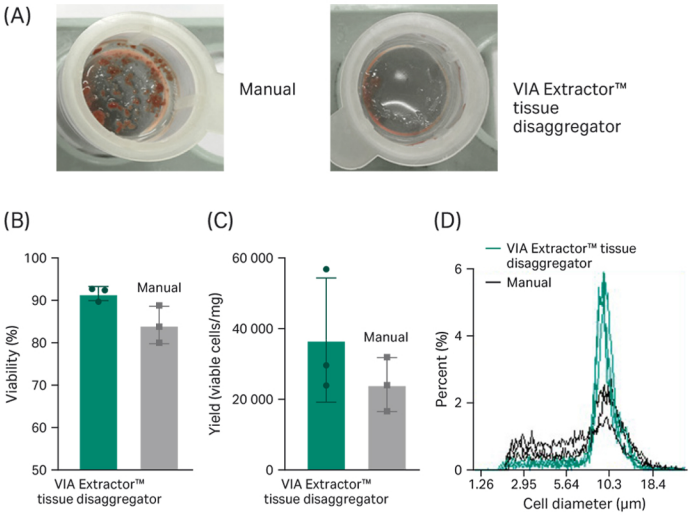

Fig 2. Three independent wild type mouse livers were halved and dissociated using either the VIA Extractor™ tissue disaggregator or traditional manual method as described above, and cell suspensions were prepared by filtering through 70 µm cell strainers. (A) The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface. Panels B, C, and D show data derived from the automated cell counter. (B) Average percentage cell viability. (C) Average yield of viable cells per mg of input tissue for dissociation. Dots show individual values. Bar shows mean of three samples. Error bars show standard deviation. (D) Histogram showing the diameter of individual cells for each sample. It can be seen that there is less debris from the VIA Extractor™ tissue disaggregator method as indicated by lower signal of particles below 8 µm.

Mouse lung disaggregation

For manual disaggregation, mouse lung tissue (100–140 mg) was minced using scalpel and scissors, washed and incubated at 37°C on rotation with 0.3% Collagenase IV for 45 minutes. For comparison, mouse lung tissue (100–140 mg) was disaggregated by placing in the Omics pouch with 0.3% Collagenase IV and running the VIA Extractor™ tissue disaggregator for 35 minutes at 200 rpm at 37°C. Again, the lung tissue was not fully dissociated into a single cell suspension using the manual technique, as is seen by the residue in the cell strainer (Fig 3A). The cell viability and yield are much better using the VIA Extractor™ tissue disaggregator (Fig 3B and 3C). There are more particles of small diameter when dissociating with the manual process compared to VIA Extractor™ tissue disaggregator, indicating the VIA Extractor™ tissue disaggregator is a gentler process resulting in less debris (Figure 3D).

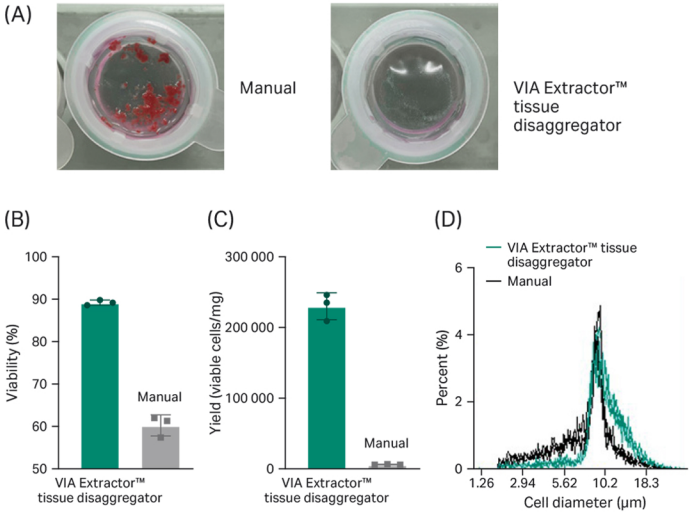

Fig 3. Three independent wild type mouse lungs were halved and dissociated using either the VIA Extractor™ tissue disaggregator or traditional mincing using scalpel blades and scissors (manual). Cell suspensions were prepared by filtering through 70 µm cell strainers. (A) The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface. Panels B, C, and D show data derived from the automated cell counter. (B) Average percentage cell viability. (C) Average yield of viable cells per mg of input tissue for dissociation. Dots show individual values. Bar shows mean of three samples. Error bars show standard deviation. (D) Histogram showing the diameter of individual cells for each sample. It can be seen that there is less debris from the VIA Extractor™ tissue disaggregator method as indicated by lower signal of particles below 8 µm.

Mouse kidney disaggregation

Mouse kidney tissue (240–300 mg) was minced using scalpel and scissors, washed and incubated at 37°C on rotation with 0.1% Collagenase IV for 30 minutes. A like sample was disaggregated by placing in the Omics pouch with 0.1% Collagenase IV and running the VIA Extractor™ tissue disaggregator for 10 minutes at 200 rpm at 37°C. The residual tissue in the cell strainer following cell filtration of the manual disaggregation incicates incomplete tissue dissociation (Fig 4A). Cell viability and yields are higher with the VIA Extractor™ tissue disaggregator (Fig 4B and 4C). With this tissue there are equal amount of small cell particles in the sample with both techniques (Fig 4D).

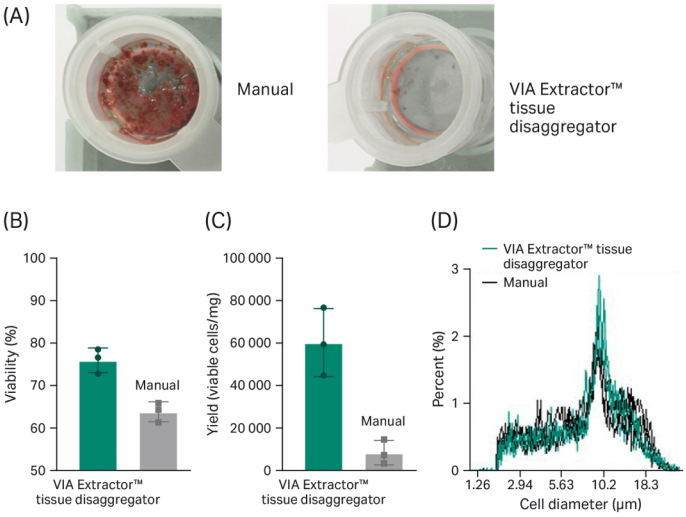

Fig 4. Three independent wild type mouse kidney pairs were dissociated using either the VIA Extractor™ tissue disaggregator or traditional mincing using scalpel blades and scissors (manual). Cell suspensions were prepared by filtering through 70 µm cell strainers. (A) The VIA Extractor™ tissue disaggregator shows more complete digestion as indicated by fewer particulates remaining on the surface. Panels B, C and D show data derived from the automated cell counter. (B) Average percentage cell viability. (C) Average yield of viable cells per mg of input tissue for dissociation. Dots show individual values. Bar shows mean of three samples. Error bars show standard deviation. (D) Histogram shows the diameter of individual cells for each sample.

This data is based on a minimum of three independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally (with the number of replicates being the same for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Sovereign House, Chivers Way, Histon, Cambridge CB24 9BZ (R&D Laboratory) during June and July 2020 and is held at this location.

Selecting a method for difficult tissues — Example: mouse brain

Brain tissue is a highly sensitive and difficult tissue from which to get viable cells after dissociation. In order to standardize this disaggregation technique to achieve the best results in downstream applications such a flow cytometry and single cell sequencing, automated mechanical methods of tissue dissociation combined with enzymatic digestion were compared.

For mouse brain (hypothalamus) dissociation, five C57BL6 mice were dissected and the hypothalamus carefully isolated. The tissues were kept in ice cold buffers and on ice to maintain the cell viability. These samples were weighed and combined. The samples were dissociated using the VIA Extractor™ tissue disaggregator method (Table 1). The Adult Brain Dissociation Kit (Miltenyi Biotec) was used and enzyme mixes were prepared according to manufacturer's protocol.

Hypothalamus samples were inserted into the Omics pouch with the help of Omics applicator. Enzyme mix from the Adult Brain Dissociation Kit (Miltenyi Biotec) were added to the compartment using luer-lock syringe and dissociated using the VIA Extractor™ tissue disaggregator at a constant speed of 200 rpm at 37°C for five minutes (Fig 5A and 5B). A complete cell suspension was achieved in this time period, as demonstrated by the lack of visible tissue pieces remaining in the cell strainer following filtration (Fig 5C).

Table 1. Amount of tissue used along with other parameters used for the dissociation

Mouse	VIA Extractor™ tissue disaggregator
Hypothalamus	56.1 mg Sample 1
Hypothalamus	105.8 mg Sample 2
Enzyme Mix	2 mL gentleMACS™ enzymes
Speed of Extractor (RPM)	200
Time to complete digestion (mins)	5
Temperature	37°C

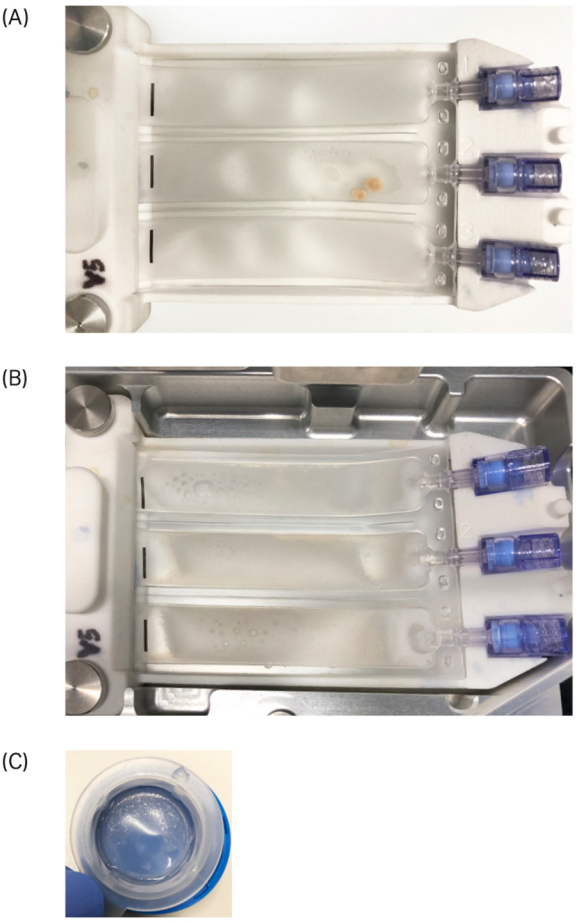

Fig 5. Preparation for murine brain dissociation using VIA Extractor™ tissue disaggregator. C57BL6 mice were dissected to isolate the hypothalamus from the rest of the brain tissue. Samples were combined from littermate controls to achieve enough material for cell isolation. (A) Assembly of Omics pouch and Omics clamp before tissue disaggregation. (B) Assembly after tissue disaggregation. (C) Cells were then passed over pre wet 70 µm cell strainers to filter out any undigested tissue material and debris. Please note that the hypothalamus sample was dissociated along with other brain samples from which the data is not included here.

Cell counting was performed using TC20™ Cell Counter (Bio-Rad) along with manual counting by Hemocytometer. Cell viability and cell yield for each sample dissociated using both instruments are shown in Table 2.

Table 2. Cell viability and yield. Cells were counted using automated TC20™ Cell Counter and compared with Haemocytometer manual counting. Trypan blue was mixed with the cell suspension in 1:1 concentration and 10 µL of sample was used in each method to count the viable cells. Cells were obtained using VIA Extractor™ tissue disaggregator

Sample	Description	Total count cells/mL	Live count Bio-Rad TC20™ cells/mL	% viability Bio-Rad TC20™ cells/mL	Haemocytometer counting cells/mL
1	VIA Extractor™ tissue disaggregator Hypo	1.83×10^7	1.37×10^7	75%	1.28×10^7
2	VIA Extractor™ tissue disaggregator Hypo	1.59×10^7	1.10×10^7	70%	1.59×10^7

Cell viability is extremely important when investigating biological pathways or transcription profiles in cells derived from brain tissue dissociation: therefore, a method that offers low impact dissociation and maximizes cell health is desirable. From this experiment, it is clear that for sensitive cells such as those in the brain, using the VIA Extractor™ tissue disaggregator for disaggregation of brain tissue provides consistently high yields with significant increase in percentage of viable cells. Additionally, dissociation was achieved in a very short time period.

This data is based on two independent experiments and/or replicate trials with the equal number of replicates in each experiment. All samples tested were treated equally and according to manufacturers' protocol and recommendations. Data was collected at University of East Anglia, Norwich Research Park, Norwich NR4 7TJ (R&D Laboratory) August 2020 and is held at this location.

Conclusion

Solid tissue dissociation using the VIA Extractor™ tissue disaggregator is a simple and efficient technique that generates viable cells in suspension for downstream analysis. The mild tissue massage offers low impact tissue dissociation to maintain cells in the best possible condition. The data described shows that the VIA Extractor™ tissue disaggregator produces higher single cell yields and viability percentages than other methods. With disaggregation taking as little as 10 minutes, the VIA Extractor™ tissue disaggregator provides the speed needed to minimize any stress-induced effects due to the processing that can interfere with downstream analysis.

Ordering information

Product	Pack size	Product code
Omics bundle (VIA Freeze™ Uno controlled-rate freezer, VIA Extractor™ tissue disaggregator, and Omics clamp)	1 of each item	29517120
Omics clamp	1	29509355
Omics pouch, 3-sample	Pack of 10	29509336
Omics applicator	Pack of 60	29509359

Related products	Pack size	Product code
GenomiPhi™ Single Cell DNA Amplification Kit	100 reactions	29108039
	25 reactions	29108107
Sera-Mag™ Select size selection and PCR clean-up reagent	5 mL	29343045
	60 mL	29343052
	450 mL	29343057
Sera-Mag™ Oligo (dT) magnetic beads	1 mL	38152103011150
	15 mL	38152103010250
	100 mL	38152103010350

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