Dissociation enzyme mixes A, B, and C

SEMI-AUTOMATED DISSOCIATION

Today, tens of thousands of individual cells from a single tissue sample or patient can be analyzed, giving researchers the opportunity to understand individual cell populations and their behavior in healthy and diseased tissue. One of the first and most critical steps in a single-cell analysis is the dissociation of tissues to yield fully dissociated but intact and viable cells.

Cytiva dissociation enzyme mixes (Fig 1) are a collection of enzymes that have been optimized for the dissociation of specific tissues. These mixes provide the user with the flexibility of performing tissue dissociation either manually or on the VIA Extractor™ tissue disaggregator and for some tissue types, such as brain, provide the additional advantage of compatibility with both warm and cold dissociation processes.

The VIA Extractor tissue disaggregator (Fig 2) is a semi-automated system that provides consistent, rapid disaggregation of solid tissue into viable single cells. It operates across a range of controlled temperatures, enabling both cold and warm dissociation processes.

This datafile presents performance data for Cytiva dissociation enzyme mixes A, B, and C optimized for mouse lung, liver, and brain tissue respectively. Performance data for dissociation enzyme mix D optimized for dissociation of kidney tissue, can be found here.

Fig 1. Dissociation enzyme mixes.

Fig 2. VIA Extractor tissue disaggregator.

Dissociation on the VIA Extractor tissue disaggregator using enzyme mixes A, B, and C

The following data were generated to demonstrate the performance of dissociation enzyme mixes A, B, and C with a semi-automated disaggregation method using the VIA Extractor tissue disaggregator. Mouse lung, liver, and brain samples were dissociated following published procedures for both cold and warm dissociation (29739555 AA, 29749726 AA, 29739368 AA).

At least three independent experiments were performed with three technical replicates consisting of three pieces of tissue of similar weight. Dissociation was carried out with the VIA Extractor tissue disaggregator using Cytiva dissociation enzyme mixes, and on the Gentle MACS Octo, using Miltenyi enzymes, and the manufacturer's protocols were followed. Crl: CD1 (ICR) mouse lungs, livers, and brains were washed in Dulbecco's (DMEM), Dulbecco's phosphate-buffered saline (DPBS) or Hank's balanced salt solution (HBSS) (Table 1). Dissociation was carried out using the enzyme volumes in Table 2 and VIA Extractor tissue disaggregator settings specified in Table 1. Tissue weights used in the experiments are shown in Figure 3. Dissociation processes are shown in Figure 4 to 6.

Table 1. Recommended sample sizes and VIA Extractor tissue disaggregator settings for dissociation

Mix and tissue type	Size/weight	Storage solution	Quench solution	Resuspension buffer	Cell strainer size	Speed	Temperature (°C)	Time
Mix A - lung	Up to 150 mg mouse lungs	DPBS	DPBS + 10% fetal bovine serum (FBS)	DPBS + 0.5% bovine serum albumin (BSA)	70 µm	200 rpm	2	60 min
Mix B - liver	Liver tissue 700 to 1000 mg	DMEM	DMEM +10% FBS	DPBS + 0.5% BSA	70 µm	60 rpm	37	22 min
Mix C - brain	1 adult brain up to 500 mg	HBSS	DPBS + 10% FBS	DPBS + 0.5% BSA	70 µm	200 rpm	2	30 min

Table 2. Enzyme volumes per sample for dissociation on the VIA Extractor tissue disaggregator with Cytiva enzyme mixes. DPBS was the buffer used for lung and brain tissue. For liver tissue, DMEM was used as a buffer.

	Mix A - lung	Mix B - liver	Mix C - brain
Reagent	Volume for 1 sample	Volume for 1 sample	Volume for 1 sample
Enzyme 3	1 mL	1 mL	-
Enzyme 4	-	-	1 mL
Enzyme 5	10 µL	10 µL	10 µL
Enzyme 6	150 µL	-	-
Buffer volume	3.84 mL	3.99 mL	3.99 mL
Total volume	5 mL	5 mL	5 mL

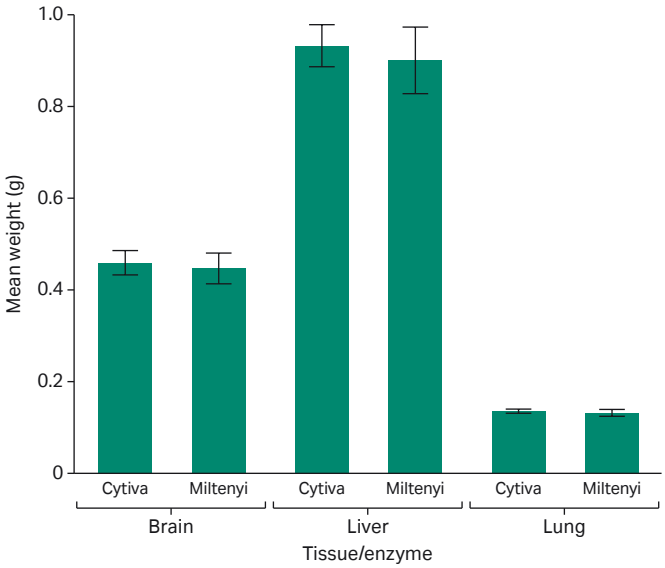

Fig 3. Mean tissue weights for each experimental replicate for dissociation on Cytiva's VIA Extractor tissue disaggregator and Miltenyi's GentleMACS Octo. For lung tissue, Miltenyi Biotech mouse lung dissociation kit was used as a comparison. For liver tissue, Miltenyi Biotec mouse liver dissociation enzyme kit was used as a comparison. For brain tissue, Miltenyi Biotec adult mouse brain dissociation enzyme kit was used as a comparison. All Miltenyi Biotec enzyme volumes were used according to manufacturer's protocols in line with recommended tissue sizes. Note: there were no significant differences in weights of tissue between enzyme mixes for each tissue type.

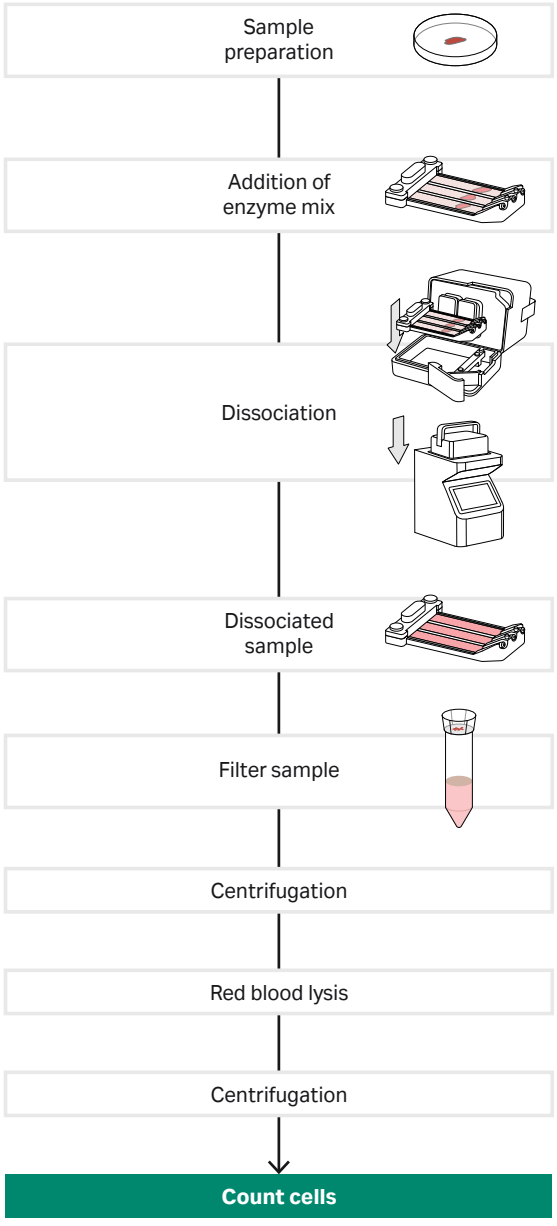

Fig 4. Tissue dissociation workflow for dissociation enzyme mixes A using the VIA Extractor tissue disaggregator. For Miltenyi enzymes, dissociation was performed using the relevant protocol on the GentleMACS Octo.

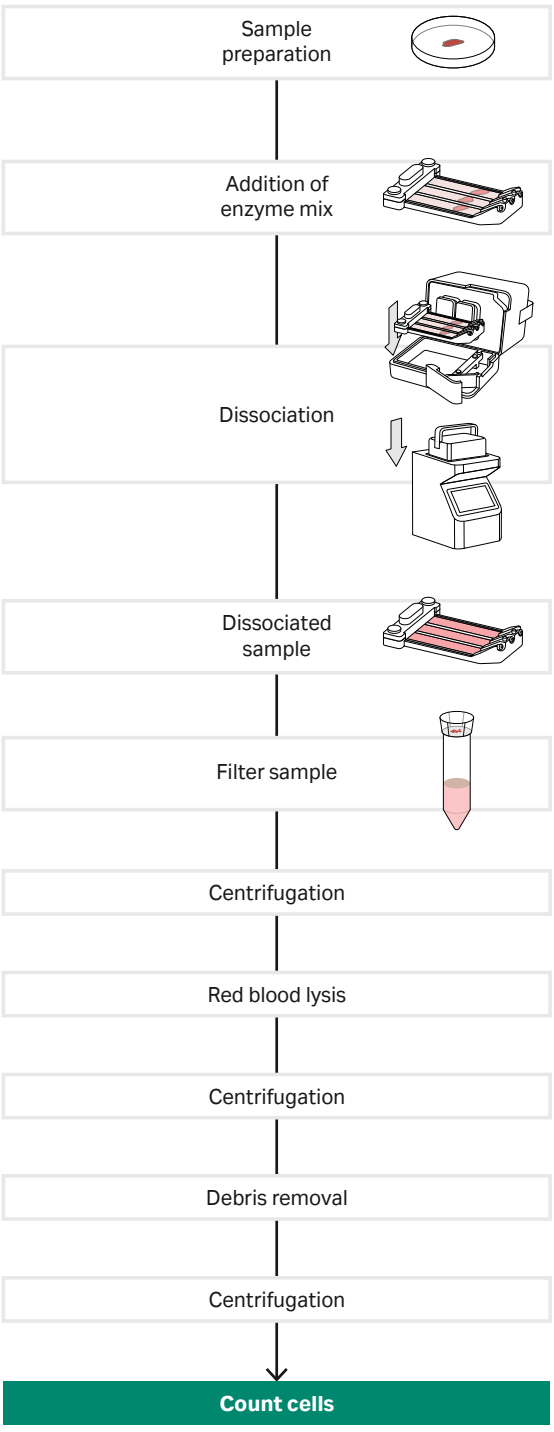

Fig 5. Tissue dissociation workflow for dissociation enzyme mixes B using the VIA Extractor tissue disaggregator. For Miltenyi enzymes, dissociation was performed using the relevant protocol on the GentleMACS Octo.

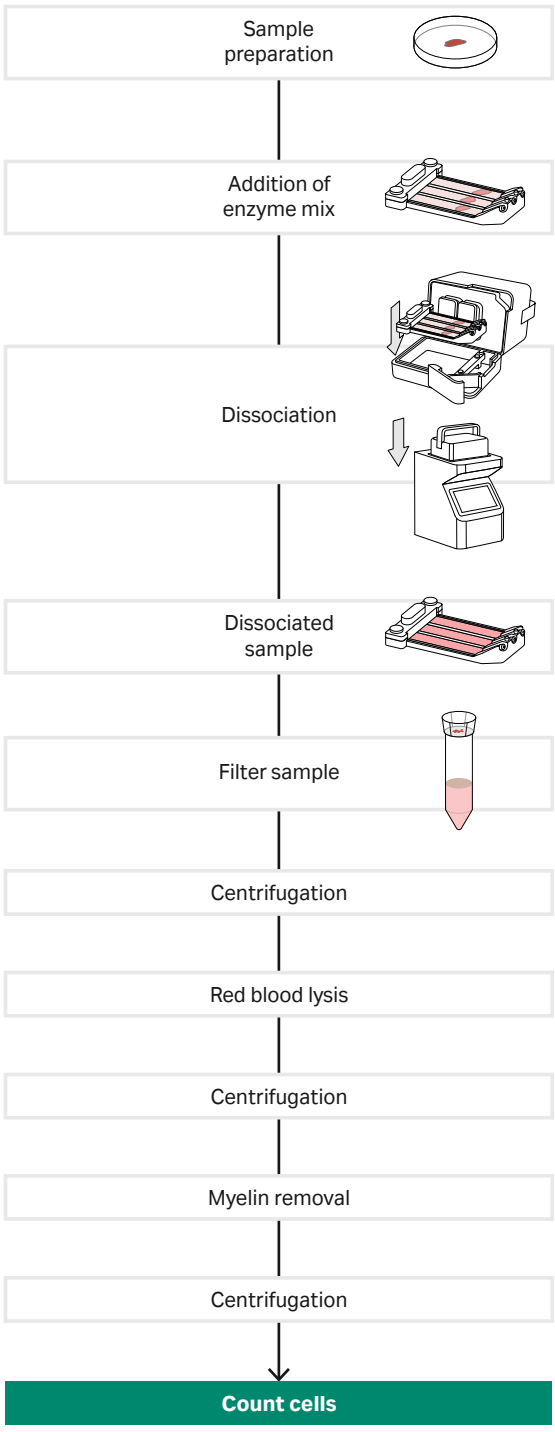

Fig 6. Tissue dissociation workflow for dissociation enzyme mixes C using the VIA Extractor tissue disaggregator. For Miltenyi enzymes, dissociation was performed using the relevant protocol on the GentleMACS Octo.

Cell straining and sample clean-up

Each tissue type was subjected to different downstream protocols as described below:

- **For lung:** Samples were subject to red blood cell lysis and resuspended in DPBS supplemented with 0.5% (w/v) bovine serum albumin (BSA). Cells were counted using a Nucleocounter™ NC-200 and VIA2-Cassettes.
- **For liver:** Samples were subject to red blood cell lysis followed by debris removal. Cells were resuspended in DPBS supplemented with 0.5% (w/v) bovine serum albumin (BSA) and counted using a Nucleocounter NC-200 and VIA2-Cassettes.
- **For brain:** Samples dissociated with Cytiva's dissociation enzyme mixes in the VIA Extractor tissue disaggregator were subject to red blood cell lysis followed by myelin removal using 27% percoll gradient centrifugation. Samples dissociated with Miltenyi's enzymes in the GentleMACs Octo were subject to Miltenyi's full method for debris removal and red blood cell lysis. All cells were resuspended in DPBS supplemented with 0.5% (w/v) bovine serum albumin (BSA) and counted using a Nucleocounter NC-200 and VIA2-Cassettes.

This data is based on three independent experiments with an equal number of replicates in each experiment. All samples tested were treated equally (with the same number of replicates for all products tested in the comparison) and according to manufacturers' protocol and recommendations. Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during 2025 and is held at this location. Where data was normally distributed, parametric tests were performed (paired t tests). All p-values are statistically significant where $p<0.05$.

Results

Dissociation enzyme mix A (lung)

Dissociation enzyme mix A on the VIA Extractor tissue disaggregator, for cold dissociation of adult mouse lung tissue, gave approximately 55% improvement in viability and a statistically significant increase in yield when compared to use of Miltenyi Biotec enzymes on the GentleMACS Octo (GMO) (Fig 7).

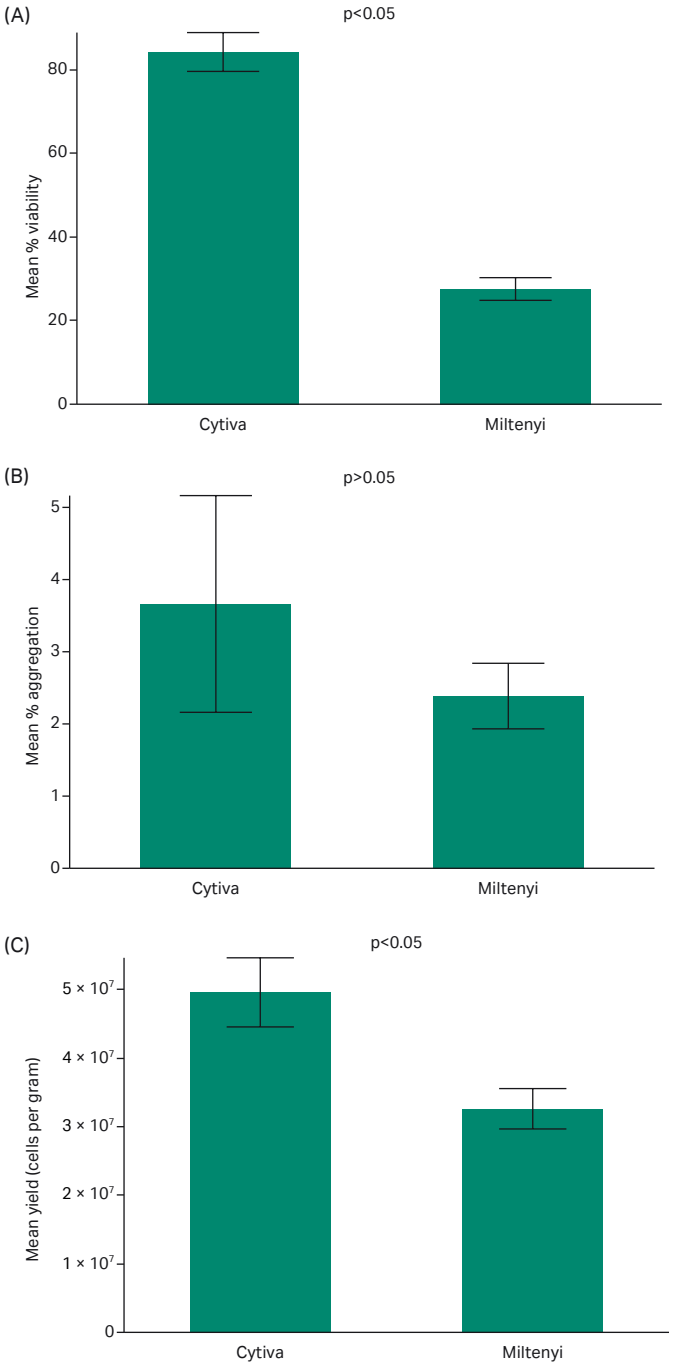

Fig 7. A comparison of Cytiva enzyme mixes A (lung-cold dissociation). Statistical analysis was performed. (A) Percentage viability for each protocol. (B) Percentage of aggregated cells for each protocol. (C) Yield of live cells per gram of tissue for each protocol.

Dissociation enzyme mix B (liver)

Liver tissue was dissociated with dissociation enzyme mix B on the VIA Extractor tissue disaggregator and gave cells that were approximately 90% viable, with a yield comparable to cells dissociated with Miltenyi's mouse liver enzyme kit (Fig 8). There was a trend for a lower percentage aggregation when using dissociation enzyme mix B on the VIA Extractor tissue disaggregator, but this did not reach statistical significance.

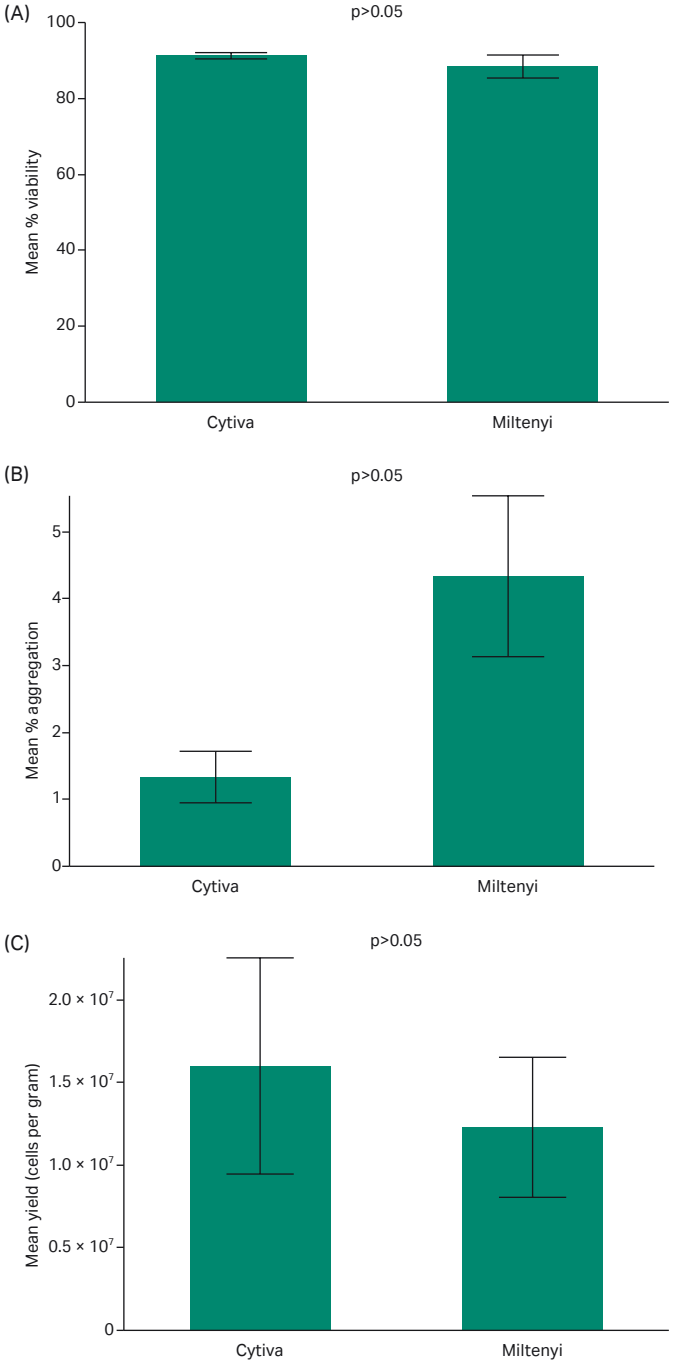

Fig 8. A comparison of dissociation enzyme mix B with liver in warm dissociation. Statistical analysis was performed. (A) Percentage viability for each protocol. (B) Percentage of aggregated cells for each protocol. (C) Yield of live cells per gram of tissue for each protocol.

Dissociation enzyme mix C (brain)

The adult mouse brain was dissociated with dissociation enzyme mix C on the VIA Extractor tissue disaggregator using cold dissociation and resulted in a 16% improvement in viability and lower aggregation when compared to samples dissociated with Miltenyi Biotec enzymes on the GentleMACS Octo (GMO) following the manufacturer's protocol (Fig 9).

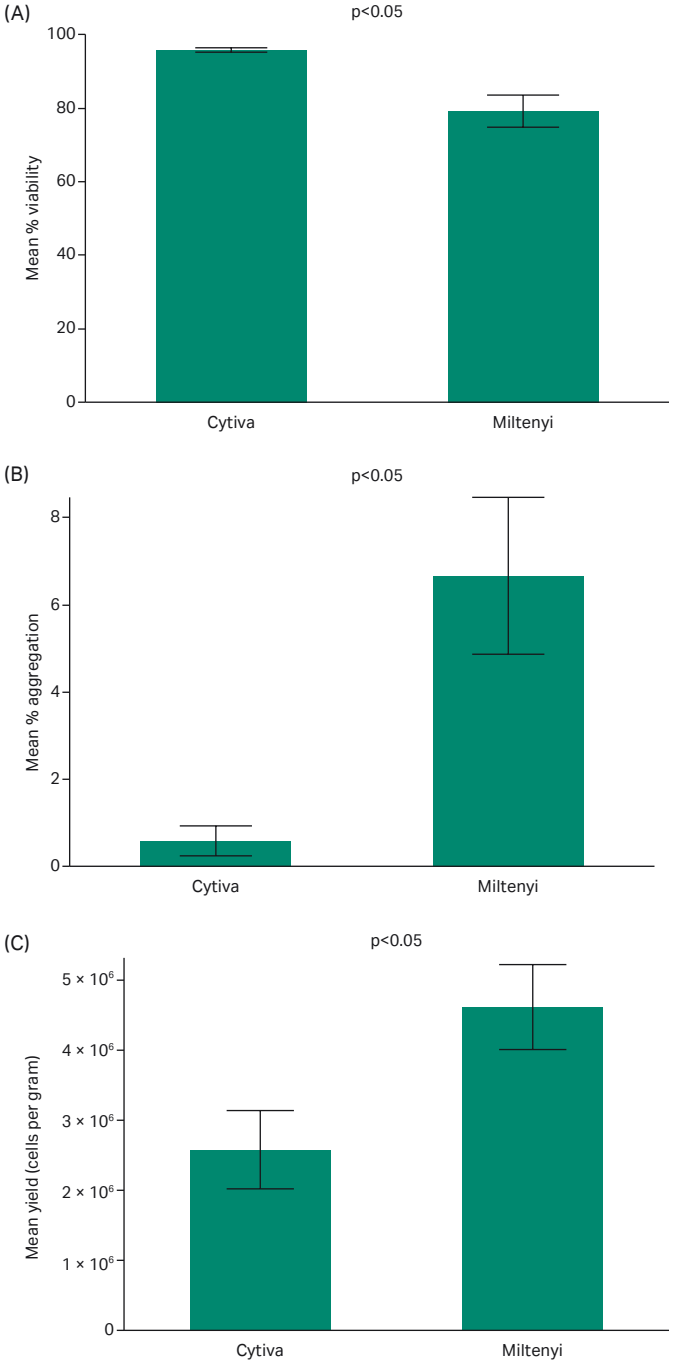

Fig 9. A comparison of dissociation enzyme mix C with brain in cold dissociation. Statistical analysis was performed. (A) Percentage viability for each protocol. (B) Percentage of aggregated cells for each protocol. (C) Yield of live cells per gram of tissue for each protocol.

Conclusion

There is increasing evidence suggesting that enzymatic dissociation combined with mechanical methods is essential for achieving complete and successful tissue dissociation. This document presents data demonstrating that Cytiva dissociation enzyme mixes A, B, and C offer several advantages for single-cell researchers. Notably, Cytiva's enzymes enable a gentler approach to mechanical dissociation of brain and lung tissue in cold semiautomated procedures, thereby enhancing cell viability for further study.

Ordering information

Product	Description	Product code
Dissociation enzyme mix A	An enzymatic kit for lung tissue dissociation. Designed for use at 4°C or 37°C, optimized for semi-automated and manual disaggregation in single-cell workflows. Each pack contains 50 reactions if used with the VIA Extractor and 25 reactions if used with manual methods.	29740243
Dissociation enzyme mix B	An enzymatic kit for liver tissue dissociation at 37°C, optimized for semi-automated and manual disaggregation in single-cell workflows. Each pack contains 50 reactions if used with the VIA Extractor and 25 reactions if used with manual methods.	29751004
Dissociation enzyme mix C	An enzymatic kit for brain tissue dissociation at 4°C or 37°C, optimized for semi-automated and manual disaggregation in single-cell workflows. Each pack contains 50 reactions if used with the VIA Extractor and 25 reactions if used with manual methods.	29740240
Dissociation enzyme mix D	An enzymatic kit for kidney tissue dissociation at 4°C, optimized for semi-automated disaggregation in single-cell workflows. Each pack contains 20 reactions.	29733434

Related products	Description	Product code
Omics bundle	VIA Extractor tissue disaggregator, VIA Freeze™ Uno controlled-rate freezer, and Omics clamp in one convenient package. VIA Freeze Uno controlled-rate freezer is a liquid nitrogen-free controlled-rate freezer.	29517120
Omics clamp	Works with Omics pouch providing a secure seal after the sample insertion whilst providing support to the bag during tissue dissociation in the VIA Extractor tissue disaggregator.	29509355
Omics pouch	A single-use, multi-compartment bag designed for dissociation of tissue into a single-cell suspension. It features three individual compartments and tubing to allow the addition of digestive enzyme solutions. Each compartment can contain up to 1.2 g of sample and 2 to 5 mL of total volume. Each pack contains 10 pouches.	29726921

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