Dissociation enzyme mixes A, B, and C

MANUAL 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 both 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.

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.

Manual dissociation using dissociation enzyme mixes A, B, and C

The following data were generated to demonstrate the performance of Dissociation enzyme mixes A, B, and C in the manual dissociation of lung, liver, and brain tissue, respectively. Published procedures were followed for both cold and warm dissociation (29739555 AA, 29749726 AA, 29739368 AA).

Lung, liver, and brain tissue from Crl: CD1 (ICR) mouse was washed in ice-cold HyClone™ Dulbecco's Modified Eagle Medium (DMEM) or HyClone Dulbecco Phosphate Buffered solution (DPBS). For details of tissue weight input and buffer solutions, please refer to Table 1 and Figure 2. For dissociation volume and enzyme composition, please refer to Table 2. A high-level overview of the tissue dissociation procedure has been depicted in Figure 3 to 5.

Fig 1. Dissociation enzyme mixes.

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

- **For lung**: Both Miltenyi Biotec and Cytiva samples were subject to red blood cell lysis and were resuspended in DPBS supplemented with 0.5% (w/v) bovine serum albumin (BSA). Cells were counted using a NucleoCounter™ NC-200 and VIA2-Cassettes (ChemoMetec).
- **For liver**: Both Miltenyi Biotec and Cytiva 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 (ChemoMetec).
- **For brain**: Cytiva samples were subject to red blood cell lysis followed by myelin removal using 27% Percoll™ gradient centrifugation. For Miltenyi Biotec samples, Miltenyi Biotec's full method was followed 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 (ChemoMetec).

All samples were paired and treated equally (with the number of replicates being the same for all products tested in the comparison). Note: The products from Miltenyi Biotec are optimized for use in automated dissociation. Performance has not been optimized by the manufacturer for use with manual methods. For Miltenyi Biotec products, Cytiva's manual dissociation protocol was followed.

Table 1. Recommended sample sizes, buffers, dissociation temperatures and times for manual dissociation

Mix and tissue type	Size/weight	Storage solution	Quench solution	Resuspension buffer	Cell strainer size	Temperature (°C)	Time
Mix A - lung	Mouse lungs, 110 to 150 mg	DPBS	DPBS + 10% Fetal bovine serum (FBS)	DPBS + 0.5% BSA	70 µm and 40 µm	37	30 min
Mix B - liver	Liver tissue, 400 to 500 mg	DMEM	DMEM + 10% FBS	DPBS + 0.5% BSA	70 µm	37	30 min
Mix C - brain	1 adult brain up to 500mg	DPBS	DPBS + 10% FBS	DPBS + 0.5% BSA	70 µm	4 and 37	30 min

Table 2. Enzyme mix volumes and composition for each sample manually dissociated with cold or warm enzyme mixes

	Mix A - lung	Mix B - liver	Mix C - brain
Reagent	Volume for 1 sample	Volume for 1 sample	Volume for 1 sample
Enzyme 3	2 mL	1 mL	-
Enzyme 4	-	-	2 mL
Enzyme 5	20 µL	10 µL	20 µL
Enzyme 6	300 uL	-	-
DMEM	-	2990 µL	-
Total volume	2.32 mL	4 mL	2.02 mL

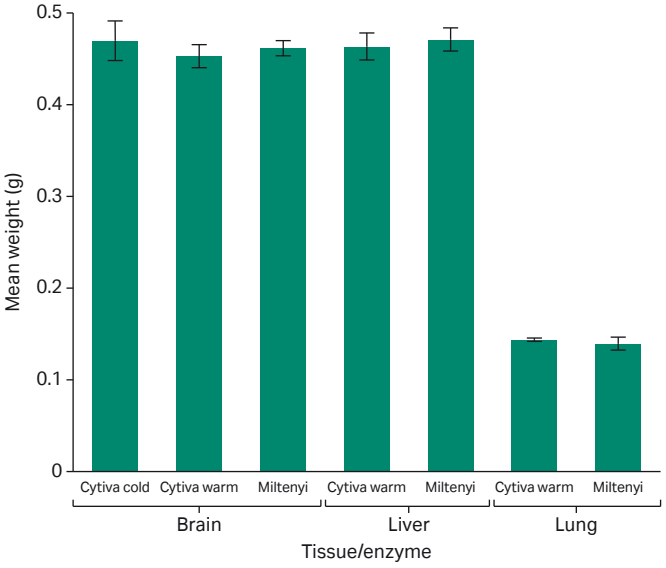

Fig 2. Tissue weights for the manually dissociated samples. For lung tissue, Miltenyi Biotech mouse lung dissociation kit was used as a comparison and performed warm. For liver tissue, Miltenyi Biotec mouse liver dissociation kit enzymes were used as a comparison and performed warm. For brain tissue Miltenyi Biotec adult brain dissociation kit (mouse) was used as a comparison and performed warm. For brain and lung, the volume of enzymes and tissue weights were as per manufacturer's instructions. For liver, 500 mg of tissue was used instead of the 750–1200 mg recommended by Miltenyi Biotec and the volume of enzymes was scaled down accordingly.

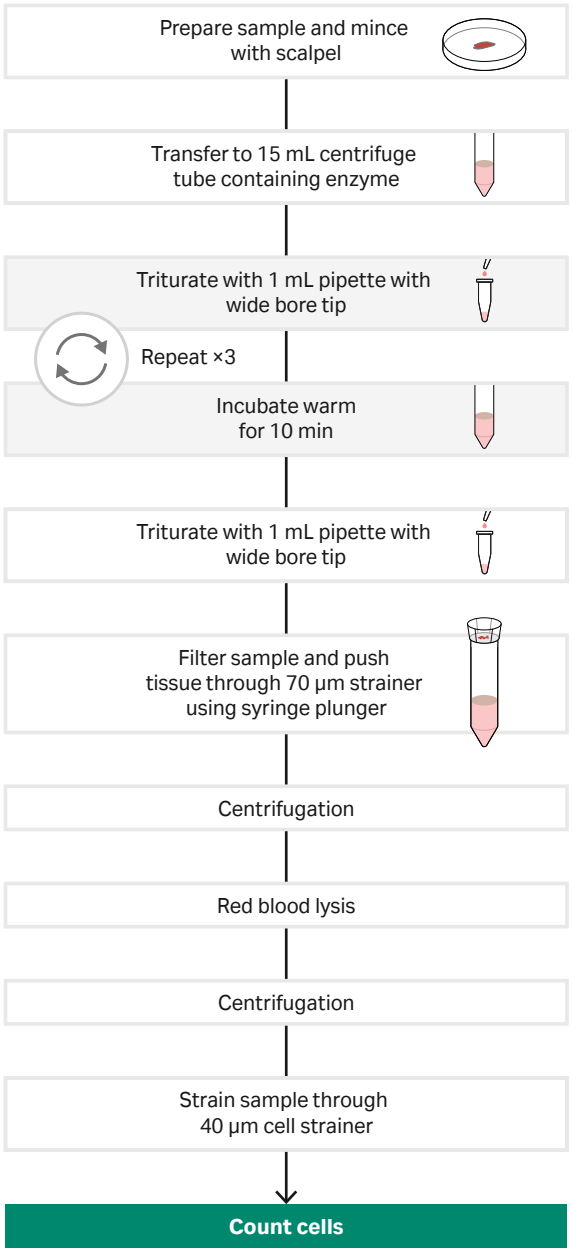

Fig 3. Manual tissue dissociation workflow for dissociation enzyme mix A (lung) and relevant Miltenyi Biotec enzyme dissociation kits.

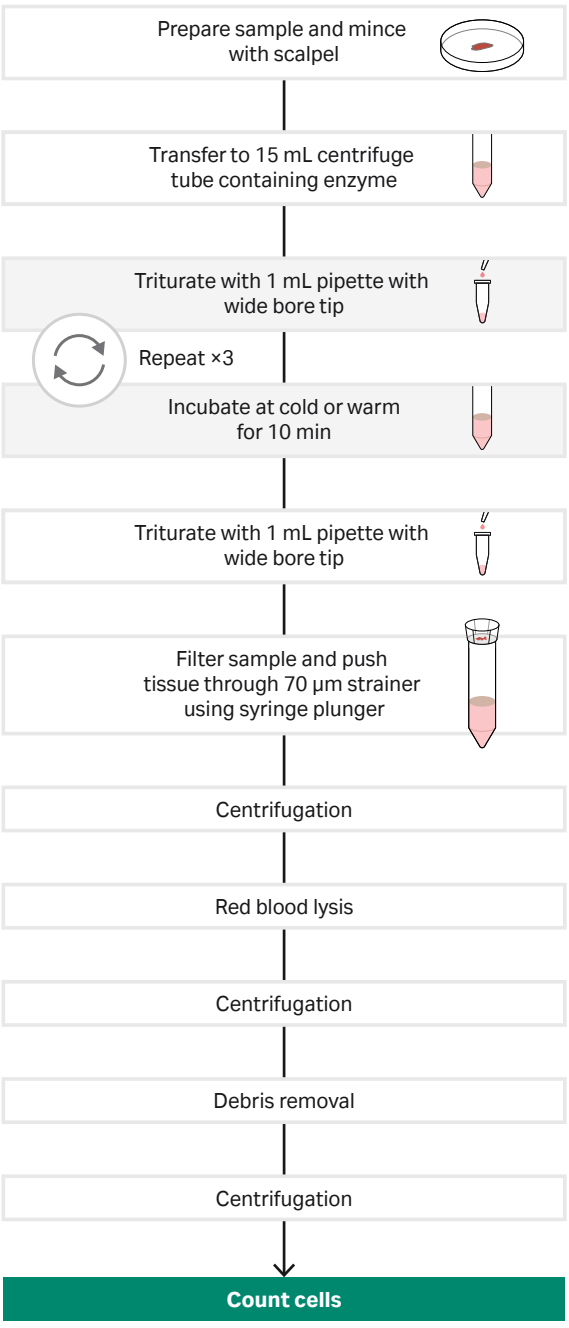

Fig 4. Manual tissue dissociation workflow for dissociation enzyme mix B (liver) and relevant Miltenyi Biotec enzyme dissociation kits.

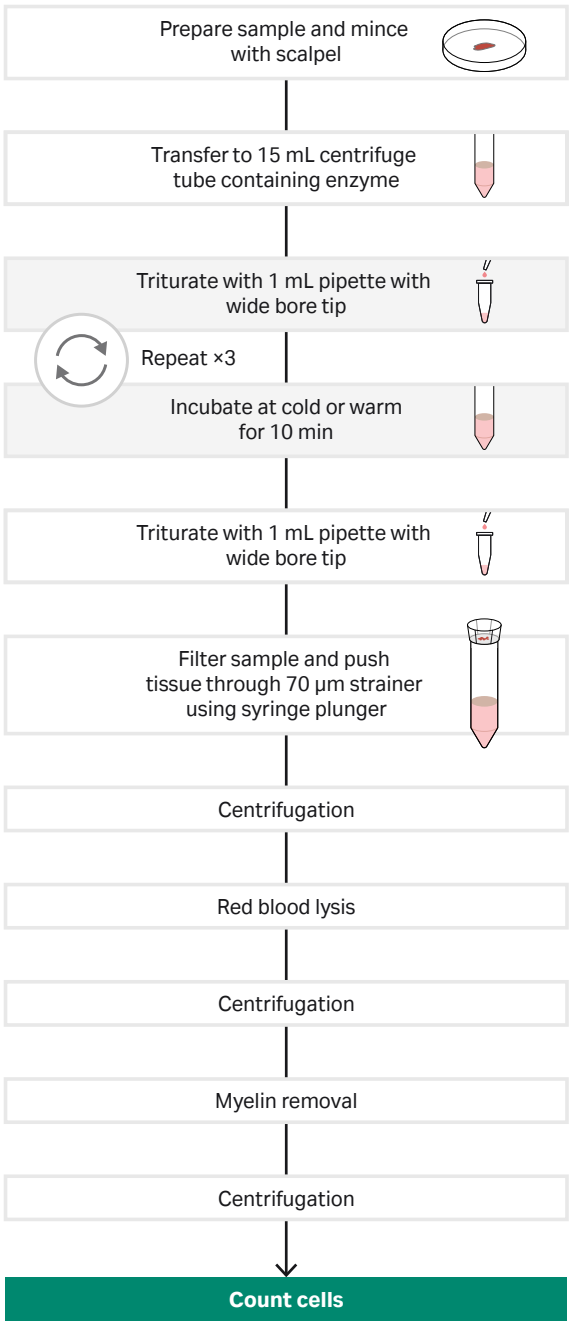

Fig 5. Manual tissue dissociation workflow for dissociation enzyme mix C (brain) and relevant Miltenyi Biotec enzyme dissociation kits.

Results

The following data demonstrates the performance of the Cytiva dissociation enzyme mixes A, B, and C for the manual disaggregation of mouse lung, liver, and brain tissue into viable cell suspensions, suitable for use in single-cell applications.

Data was collected at Cytiva, Maynard Centre, Cardiff, UK (R&D Laboratory) during 2025 and is held at this location. Two-tailed paired t-test and one-way ANOVA with Dunnett's multiple comparisons test were performed where applicable. All p-values are statistically significant where $p<0.05$. This data is based on three independent experiments with three paired technical replicates in each experiment.

Dissociation enzyme mix A (lung) used at 37°C

Here, we demonstrate that Cytiva's dissociation enzyme mix A, for lung dissociation offers improved viability and a trend for improved yield when used in warm, manual dissociation when compared with Miltenyi Biotec enzymes. It should be noted that Miltenyi Biotec enzymes are optimized for use in automated dissociation on the Miltenyi Biotec Gentle MACS Octo instrument and not for use in manual dissociation.

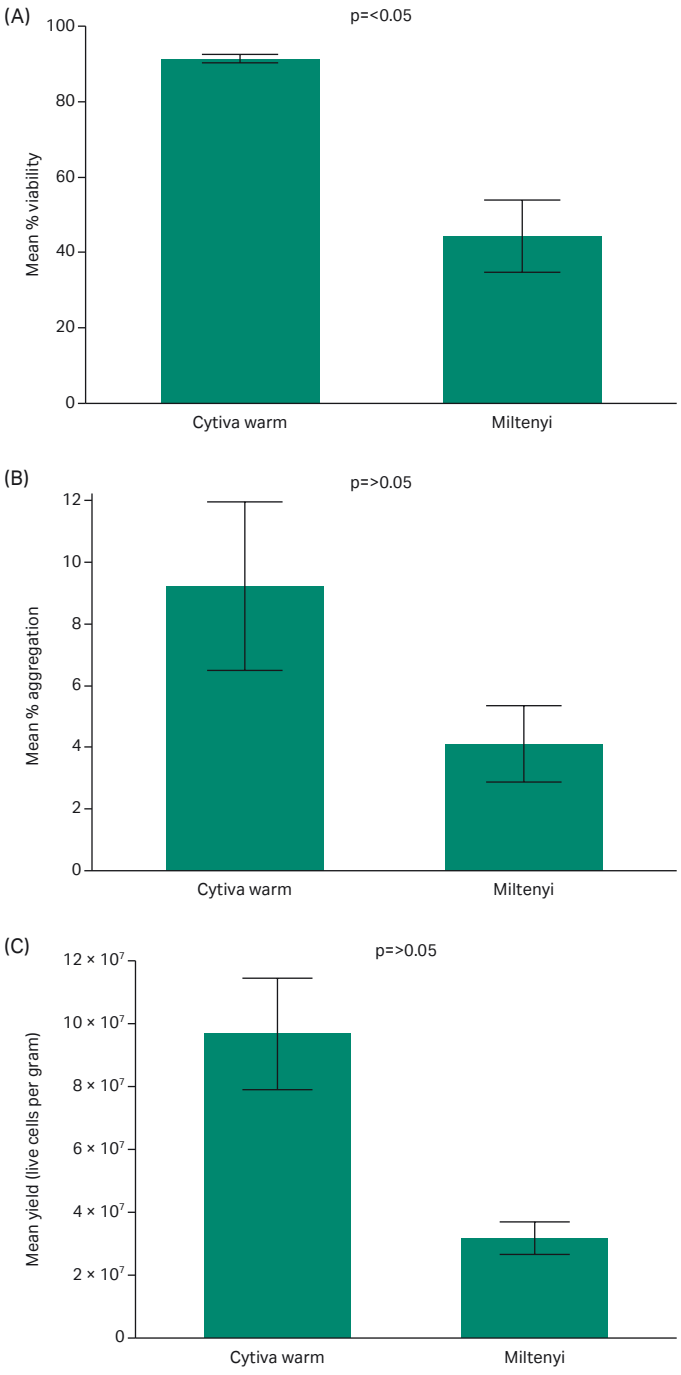

Fig 6. Comparison of data using Cytiva enzyme mix A (lung) and Miltenyi Biotec Mouse lung dissociation kit in warm manual dissociation. (A) Percentage viability for each protocol. (B) Percentage of aggregated cells for each protocol. (C) Yield of live cells per gram of tissues for each protocol

Dissociation enzyme mix B (liver) used at 37°C

Here, we demonstrate that Cytiva's dissociation enzyme mix B, for liver dissociation offers viability, yield and percentage aggregation as good as Miltenyi Biotec enzymes when used in warm, manual dissociation. It should be noted that Miltenyi Biotec enzymes are optimized for use in automated dissociation on the Miltenyi Biotec Gentle MACS Octo instrument and not for use in manual dissociation.

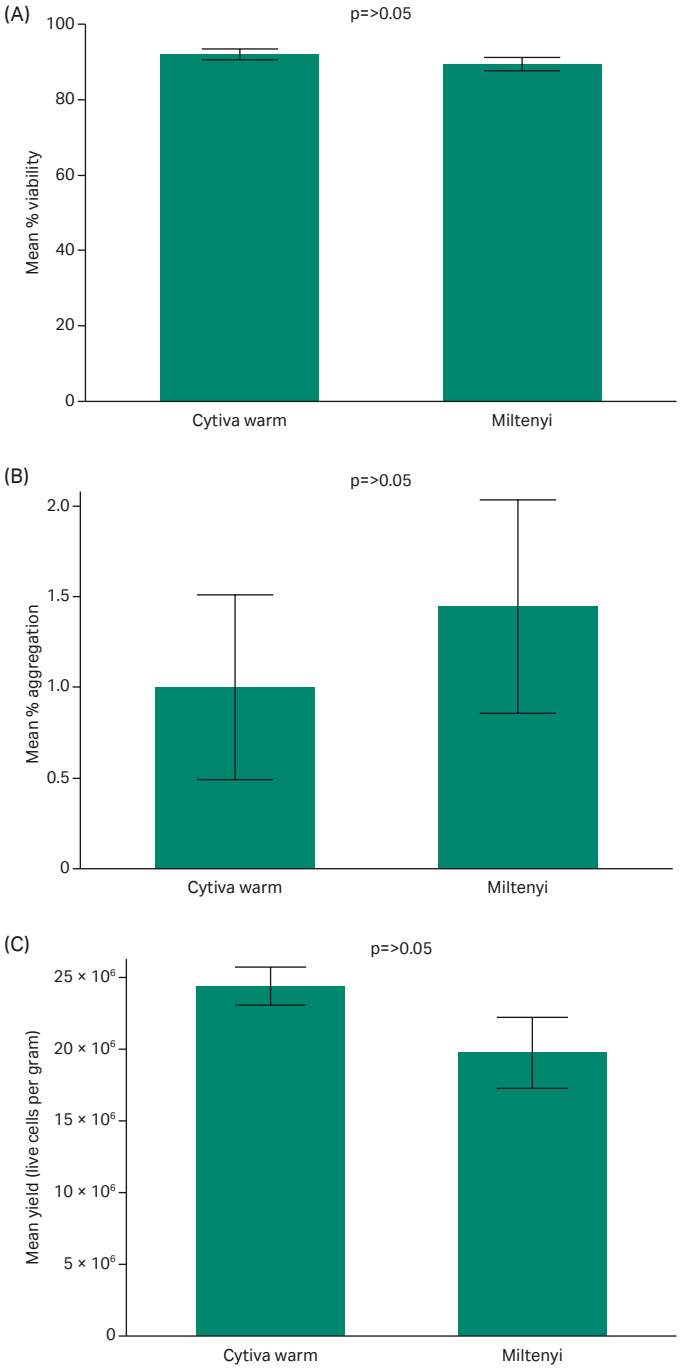

Fig 7. Comparison of data using Cytiva enzyme mix B (liver) and Miltenyi Biotec Mouse liver dissociation kit in warm, manual dissociation. (A) Percentage viability for each protocol. (B) Percentage of aggregated cells for each protocol. (C) Yield of live cells per gram of tissues for each protocol.

Dissociation enzyme mix C (brain) used at 4°C and 37°C

Here, we demonstrate that Cytiva's dissociation enzyme mix C, for brain dissociation offers greater than 90% viability when used in warm and cold manual dissociation and yield and percentage aggregation as good as Miltenyi Biotec enzymes. It should be noted that Miltenyi Biotec enzymes are optimized for use in automated dissociation on the Miltenyi Biotec Gentle MACS Octo instrument and not for use in manual dissociation.

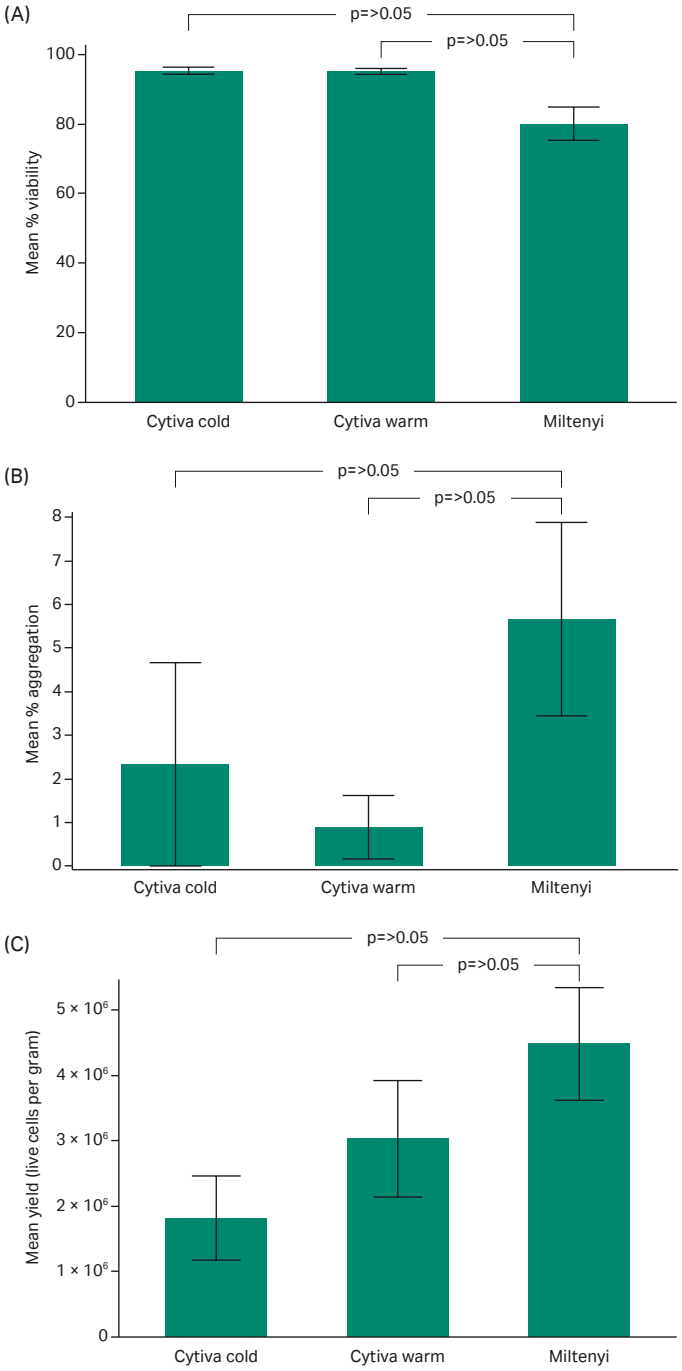

Fig 8. Comparison of data using Cytiva enzyme mix C (brain) and Miltenyi Biotec adult mouse brain dissociation kit in warm and cold manual dissociation. Bar lines represent the standard error of the mean. (A) Percentage viability for each protocol. (B) Percentage of aggregated cells for each protocol. (C) Yield of live cells per gram of tissues for each protocol.

Conclusion

There is growing evidence to support enzymatic dissociation, in combination with mechanical aid, is crucial to achieve a complete and successful dissociation. Here we present data that demonstrates Cytiva dissociation enzyme mixes A, B, and C work effectively in manual dissociation providing flexibility for researchers in single-cell workflows and applications. Importantly, Cytiva's enzyme mix A, for lung, gives improved viability and yield in comparison to Miltenyi Biotec enzymes using Cytiva's manual protocol. Cytiva dissociation enzyme mixes therefore demonstrate optimal cell quality with manual dissociation as well as with automated dissociation when used with the VIA Extractor tissue disaggregator.

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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