Dissociation enzyme mix D (kidney)

ENZYME DISAGGREGATION FOR OPTIMAL CELL VIABILITY WITH KIDNEY TISSUE

Single-cell RNA sequencing has rapidly become the method of choice to investigate cellular transcriptomic heterogeneity among cell populations. To get the best quality data possible from single cell RNA sequencing it is essential that the tissue is extracted, handled, and processed quickly and with care to obtain viable, single, non-aggregated cells.

Manual tissue dissociation is long, laborious and affects cell viability. It is therefore vital to choose equipment such as the VIA Extractor™ in association with dissociation enzymes mixes to speed up the single cell workflow yet at the same time be gentle on the cells and preserve the original cell state as much as possible.

Fig 1. The dissociation enzyme mix D (kidney) kit.

Key features and benefits

Developed for use with the VIA Extractor tissue disaggregator, the dissociation enzyme mix D (kidney) (the Cytiva kit) comprises two enzymes and a buffer.

Dissociation at cooler temperatures using the Cytiva kit and VIA Extractor tissue disaggregator:

- Improves cellular viability
- Reduces cellular aggregation
- Reduces cell stress in comparison to warm enzymes.

The VIA Extractor tissue disaggregator can sustain a cold temperature in a controlled manner making it ideal for dissociation at cooler temperatures thereby reducing cell stress and minimizing cell death and improving the transcriptional profile of the single cells.

Fig 2. The VIA Extractor tissue disaggregator.

Dissociation enzyme mix D (kidney) performance

The following data was generated to demonstrate the effectiveness of the Cytiva kit for disaggregation of mouse kidney into a viable cell suspension that is suitable for use in single-cell RNA sequencing.

Mouse kidneys were obtained from six female Crl:CD1 (ICR) mice and washed in ice cold Dulbecco's phosphate buffered saline (DPBS). Connective tissue was removed and kidneys were dissociated on the VIA Extractor tissue disaggregator as in Fig 3 and Table 1.

Table 1. Tissue weights and enzyme mix volumes for each sample dissociated with cold enzyme mix. Note that two experiments were performed with two separate users on two separate occasions.

User	Mouse	Kidney (mg)	Volume of enzyme mix
1	1	299	5 mL
1	2	245	5 mL
1	3	267	5 mL
2	1	226	5 mL
2	2	247	5 mL
2	3	239	5 mL

For cold dissociation using the Cytiva kit on the VIA Extractor tissue disaggregator, the Cytiva kit procedure was followed.

All cell suspensions were passed through 100 µm cell strainers and subjected to red blood cell lysis. Following red blood cell lysis, cells were resuspended in DPBS supplemented with 0.4% (w/v) bovine serum albumin (BSA) and 0.1 mM ethylenediaminetetraacetic acid (EDTA) and filtered through 70 µm then 40 µm cell strainers. Cells were counted using a Nucleocounter NC-200™ and VIA2-Cassettes™ (ChemoMetec). All t-tests were performed in JMP® Statistical Discovery software by SAS.

Table 2. Temperature, speed and time conditions for dissociation enzyme mix D (kidney). Note the program temperature is set at 2°C to allow the VIA Extractor tissue disaggregator to cool to 4°C.

Program speed	200 rpm
Program time	15 min
Program temperature	2°C

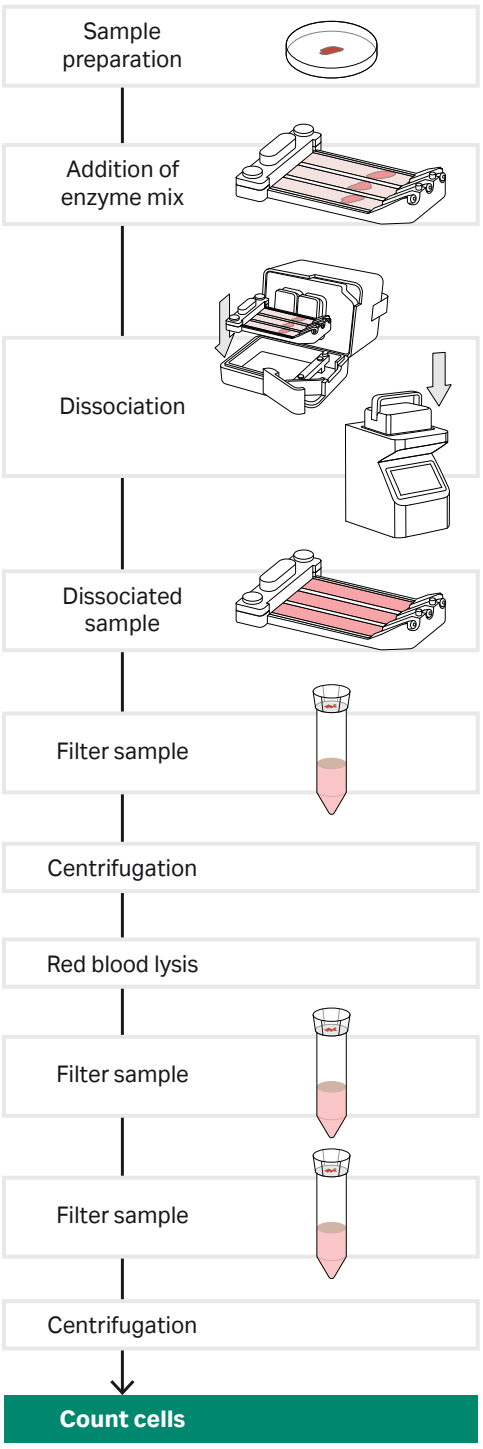

Fig 3. Tissue dissociation workflow for dissociation enzyme mix D (kidney) using the VIA Extractor tissue disaggregator. Note the procedure had three sample filter steps. The first with 100 µm cell strainer, the second with 70 µm cell strainer and the third with 40 µm cell strainer.

Results

Use of the Cytiva kit with VIA Extractor tissue disaggregator results in high viability and few aggregated cells. This is important to increase capture of viable single cells rather than multiplets in single cell sequencing.

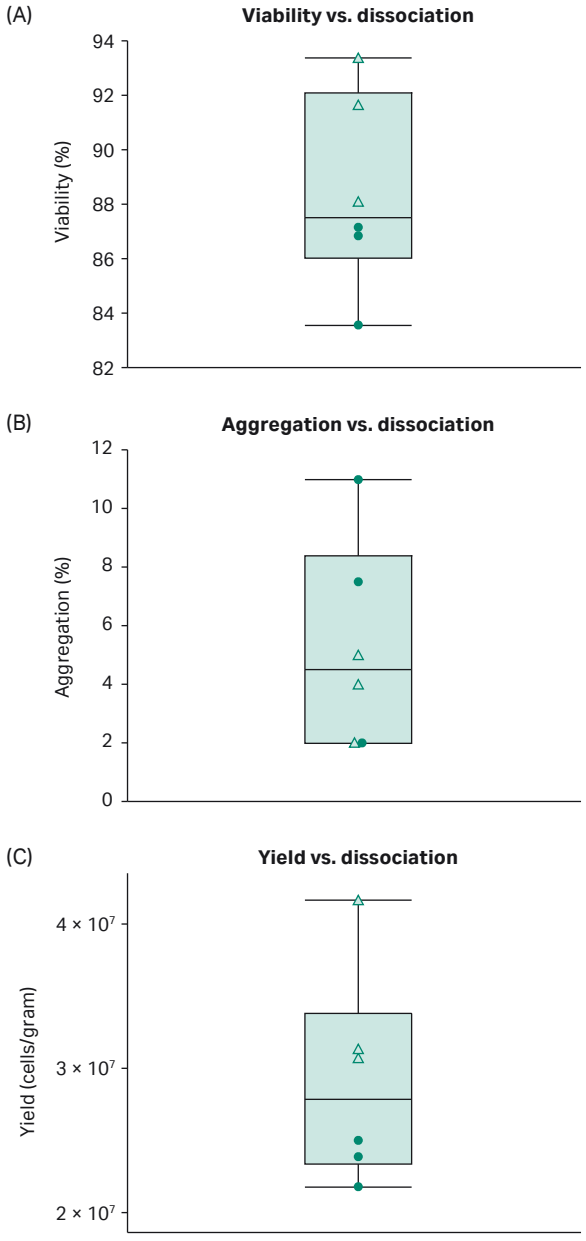

Fig 4. Data show that the dissociation enzyme mix D (kidney) when used in conjunction with the VIA Extractor tissue disaggregator gives cell viability greater than 80%, gives less than 10% aggregates and yields over 2 × 10^7 cells per gram. User 1 is represented by circles and user 2 represented by triangles.

Conclusion

There is growing evidence to support that cold enzymatic dissociation is beneficial to preserve cell state and reduce cell stress during the dissociation process. Here we present data that demonstrates Cytiva's kit has multiple benefits for the single cell researcher: reduces clumping of cells and improves cell viability. Cytiva's kit may also reduce RNA degradation, preserve the transcriptome and allow detection of cells difficult to sequence due to high RNase content, read more.

Ordering information

Product	Description	Product code
Dissociation enzyme mix D (kidney)	1 pack × 20 reactions	29733434

Related product	Description	Product code
Omics bundle	Includes VIA Extractor tissue disaggregator, VIA Freeze™ Uno controlled-rate freezer, and Omics clamp	29517120

cytiva

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For local office contact information, visit cytiva.com/contact

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