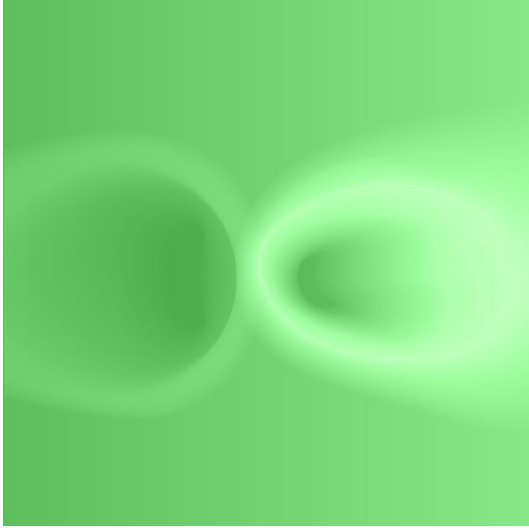
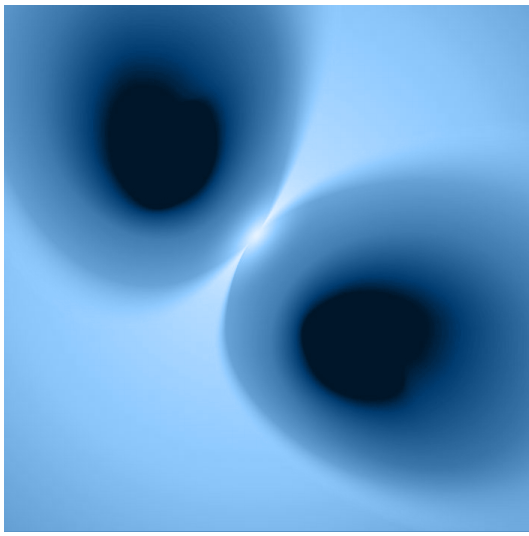




Automated Counting of Isolated Nuclei

Advances in Quality Control Methods for Nuclei Sample Prep

DeNovix®

What You'll Learn

Isolating nuclei is critical for single nuclear workflows such as RNA-seq and ATAC-seq. Ensuring that non-clustered, debris-free samples are obtained is crucial to successful library preparation. Even when debris does not affect library preparation itself, **it is important to count nuclei accurately for single-cell analysis.**

The resources in this eBook will give you a detailed look at our recommended solution for the automated counting of isolated nuclei, **the DeNovix CellDrop™ Automated Cell Counter.** We'll explore how the CellDrop's DirectPipette™ technology, dedicated nuclei counting software applications, and fluorescence capabilities combine to provide accurate nuclei count data.

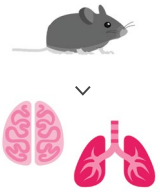
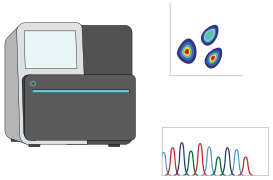
Contents

INTRODUCTION

Why Use a Cell Counter for Nuclei Quality Control?

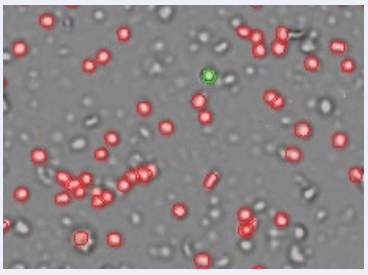
Prior to downstream applications like single-cell sequencing, it is crucial to perform quality control analyses for single nuclei sample preparation. Using an automated cell counter along with fluorescence viability dyes improves the accuracy and consistency of nuclei counting, standardizing protocols and removing user-to-user variability.

The CellDrop Automated Cell Counter is an efficient and reliable method for quantifying nuclei, measuring extraction efficiency, and visualizing debris in a sample. As an added benefit for both the environment and costs, the instrument can analyze samples without requiring disposable plastic slides.

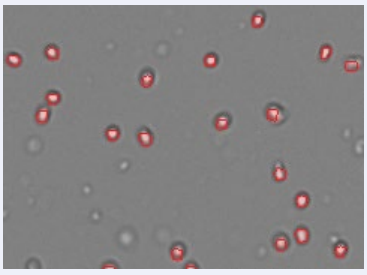
Tissue	Nuclei Isolation	Count with CellDrop	Downstream Applications
			
• Fresh Tissue • Frozen Tissue • Fixed Tissue	User-Formulated Buffer	• Nuclei concentration • Nuclei extraction efficiency • Cluster reports • Debris visualization	• RNA-seq • ATAC-seq • Flow Cytometry

Example Images: Isolated nuclei (red) & intact cells (green) counted with CellDrop + AO/PI dye

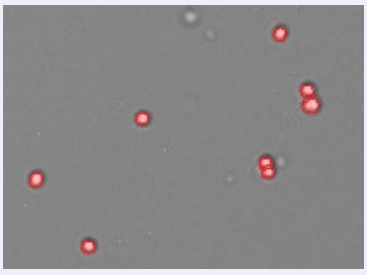
Nuclei extractions have been successfully tested on Frozen Mouse Kidney, Frozen Mouse Prostate, Frozen Rat Lung, Fresh Mouse Kidney, Fresh Mouse Lymph Node, Fresh Mouse Lung, and Fresh Mouse Brain. This list is not intended to be exhaustive, but simply to highlight testing completed in our lab. Many other tissues and methods are possible and have been successfully measured by our customers.



10X Genomics™ Nuclei Isolation Kit
Sample from Mouse Kidney



Miltenyi Biotec Nuclei Isolation Buffer
Sample from Rat Lung



User-Formulated Nuclei Isolation Buffer
Sample from Mouse Lymph Node

TECHNICAL NOTE 216

Counting Isolated Nuclei Using CellDrop

The pre-installed Nuclei AO/PI (left) and Nuclei Trypan Blue (right) apps on the CellDrop Automated Cell Counter are optimized with protocols for isolated nuclei counts.

In this technical note, learn how to count isolated nuclei on the CellDrop.

Nuclei AO/PI

Nuclei Trypan Blue

Nuclei Isolation Procedure & Considerations

Nuclei were isolated from HEK293T cell cultures according to the 10X Genomics® protocol for the "Isolation of Nuclei from Single Cell Suspensions. CG000124 Rev D." Before lysis, cell density and viability were assessed using the standard CellDrop AO/PI protocol to confirm a minimum of ~2.5 million cells/mL at >90% viability.

Nuclei Isolation Procedure & Considerations (cont'd)

With the basic performance established, isolations were performed from fresh and frozen tissue using Miltenyi Biotec's Nuclei Extraction Buffer (130-128-024) and gentleMACs™ Dissociator. Images in this technical note have been selected from mouse liver samples, but extractions have been tested on mouse frozen mouse lung, prostate, kidney, brain, and liver, fresh mouse prostate, brain, and liver, and frozen rat lung.

Keeping the nuclei at 4°C throughout the protocol is important for high quality extractions. To ensure high yields, it is recommended to keep samples on fresh ice and work as quickly as possible. While not necessary for high yields, the gentleMACS™ Octo Coolers (130-130-533) were used in this procedure to keep nuclei at optimal temperatures throughout the extraction process.

The CellDrop count results showed a high extraction efficiency (>90%), but also contained cellular debris as is expected of whole organ isolations.

While the gentleMACS™ Dissociator system and filtration steps reduce debris, different tissue types may be more challenging to work with. Tissues from the brain or heart, for example, typically contain more debris than liver and lung samples.

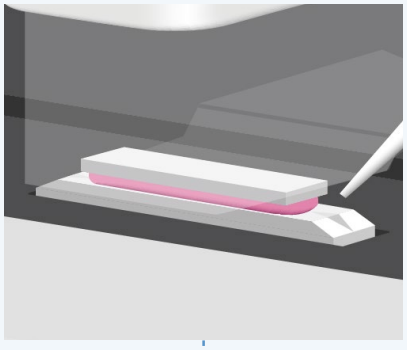
An optional step is to remove debris from samples using kits such as the Miltenyi Anti-Nuclei Microbeads.

Minimizing debris and large clusters is important for the downstream workflow of single-cell sequencing. These can clog the fluidic chips, resulting in low quality libraries or failed sequencing experiments. Refer to the manufacturer's protocol if large clusters of nuclei are observed. Similarly, removing intact cells that did not lyse during the procedure is also recommended.

CellDrop DirectPipette™ Technology

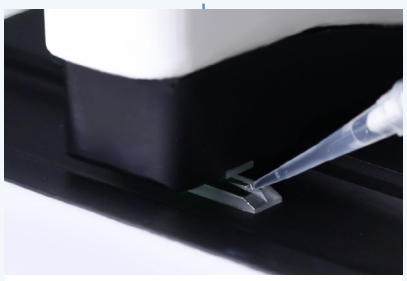
The unique DirectPipette™ technology of CellDrop Automated Cell Counters enables counting without disposable slides, which reduces plastic waste and costs. The variable chamber volume allows counting volumes of between 5 µL and 40 µL of sample.

In addition, CellDrop is also compatible with common disposable plastic or reusable slides to allow the user to both quantify the nuclear isolation on the CellDrop and transfer the same slide to a microscope with a higher magnification for nuclear integrity analysis.



How it Works: The Sample Chamber

- Two permanent, optical grade sapphire surfaces
- Sample is held in place by surface tension
- Three software-selectable chamber heights enable:
  - Widest dynamic range: 7 x 10^2 – 2.5 x 10^7 cells/mL
  - Cell size range: 4 – 400 µm

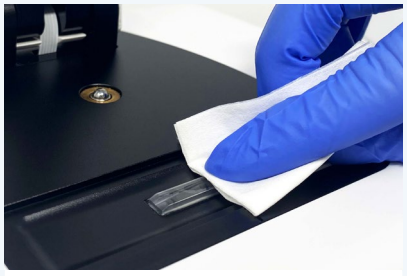


1 Load Sample

Load cell suspension into sample chamber. Sample can be loaded from either side of the arm.

2 Count & Analyze

Press the Count button, and let the CellDrop do the work! Count data will appear in 3 – 8 seconds.



3 Wipe Clean

After wiping the surfaces with a dry lab wipe, the CellDrop is ready for the next sample.

Counting Nuclei with AO/PI Stain

Acridine Orange and Propidium Iodide (AO/PI) can be used to determine the success of nuclei isolations. In traditional cell viability testing, the AO/PI dye combination stains live cells so they fluoresce green and dead cells fluoresce red. However, the stain will also label successfully isolated nuclei red and any remaining intact cells green. This allows the user to calculate the residual intact cells that carry over as a percent of the total counted and determine if the experimental workflow can proceed.

As the nuclear pore complex will allow passive diffusion up to 30-60 kDa, both AO and PI (~0.6 kDa) freely pass into the nucleus and display a red signal due to a FRET interaction between the two fluorophores. Minimizing the number of intact cells in isolation is important, so accurately enumerating the intact cells with AO/PI can improve quality control and improve consistency in the results of downstream workflows.

Software Results & Image Analysis

The following count (Figure 1) was made using the CellDrop Nuclei AO/PI App to analyze nuclei isolated from a mouse kidney sample. The CellDrop quantifies isolated nuclei (stained red) and the intact cells (stained green) to calculate the nuclei/mL concentration and nuclei extraction efficiency for each sample. Measurements are readily presented to the user on the results screen with other relevant sample data. Images and sample data are automatically saved to the instrument and can be recalled for review at any time.

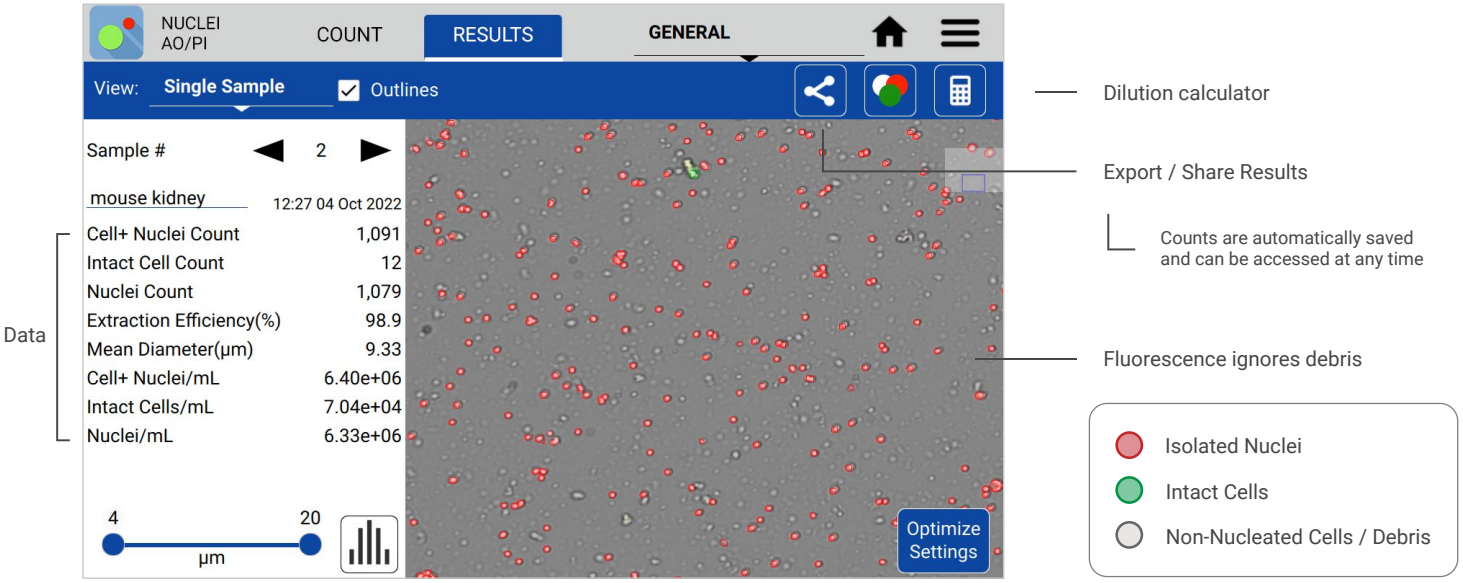


Figure 1: CellDrop isolated nuclei result image. Nuclei are stained red while leftover intact cells are stained as green. Nuclei were isolated using the Miltenyi Biotec gentleMACS™ Tissue Dissociator and Nuclei Extraction buffer.

Software Results & Image Analysis cont'd

A common requirement for high quality single nuclei sequencing library preparation is the need for cluster-free, monodispersed samples with accurate diameter assessment. Information such as nuclei/mL density, level of intact cell contamination via the extraction efficiency, and mean diameter size is readily accessible in the Results screen. Users can easily access advanced cluster analysis (Figure 2) on a per sample basis by switching to the Graphs view in the Results screen and selecting the Cluster Size bar graph by the dropdown menu.

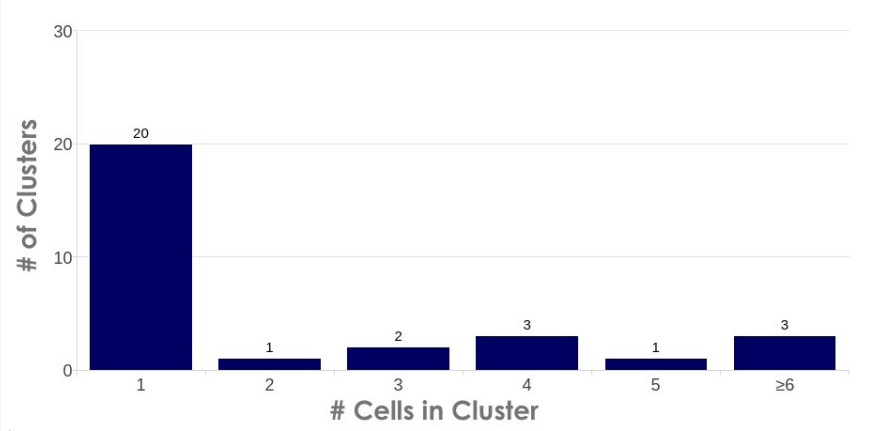


Figure 2: Count cluster data can be found in the CellDrop Graphs view. The software allows the user to visualize the cluster size and the frequency of up to six different cluster sizes for each count on the device.

Debris and Purification

The CellDrop fluorescence mode offers advantages, such as easily distinguishing background debris from nuclei, to increase the accuracy of results. However, large amounts of debris left in the sample might create challenges in downstream applications. Some tissue types may benefit from filtration or purification before moving onto the sequencing step.

Figure 3 compares the same sample (isolated nuclei from mouse brain tissue) counted on the CellDrop Nuclei AO/PI App before (3A) and after (3B) purification. In 3A, the CellDrop software ignores debris and red blood cells to provide an accurate count of isolated nuclei and intact cells. In 3B, the same sample was analyzed again after nuclei enrichment using Miltenyi's Anti-Nuclei Microbeads to remove debris.

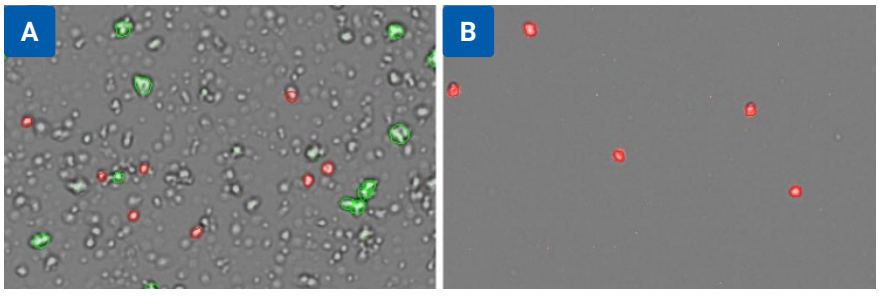


Figure 3

3A: Isolated nuclei from mouse brain tissue counted on the CellDrop FL Nuclei AO/PI app.

3B: Same sample after purification with Anti-Nucleus Microbeads (Miltenyi Biotec).

Counting Nuclei with Trypan Blue Stain

Where dual fluorescence instrumentation is not available, it is also possible to analyze the success of a nuclear isolation using Trypan Blue. **DeNovix recommends using fluorescent assays for quantifying isolated nuclei where possible due to the increased accuracy ensured by the clear differences in green and red signals.** It should be noted that counting debris-laden samples using Trypan Blue can increase the number of erroneous counts either with an automated cell counter or by manual count.

Figure 4 shows HEK293T cells stained with Trypan Blue and counting using the CellDrop Trypan Blue app. The Trypan Blue dye enters all of the successfully extracted nuclei (circled in red) and stains them, giving them a dark appearance. The intact cells (circled in green) that were not successfully lysed exclude the Trypan Blue dye and appear white.

The numbers of nuclei and intact cells are used to quantify the extraction efficiency for a sample to ensure that enough nuclei are available for the subsequent experiments.

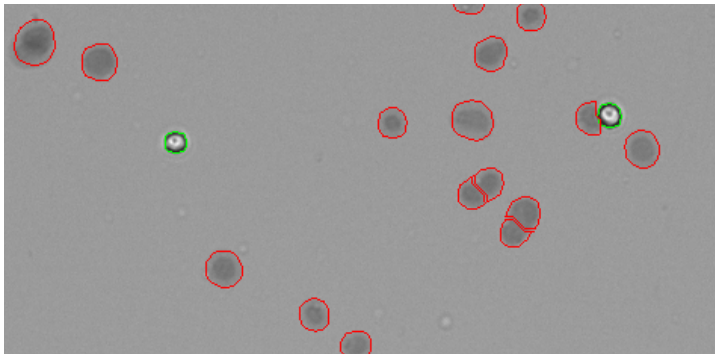


Figure 4: Isolated nuclei counted with Trypan Blue on the CellDrop. The CellDrop does an excellent job of recognizing isolated nuclei in a carefully purified isolation of HEK293T cells. The nuclei are stained dark, indicating uptake of the Trypan Blue dye (circled red), while intact cells exclude the dye and remain bright (circled green).

Conclusion

Quality control is an important step in preparing nuclei suspensions for downstream applications. Single-cell sequencing procedures such as those employed by 10X Genomics® rely on isolated nuclei for the technology to appropriately detect expression differences in a cellular population. The CellDrop Automated Cell Counter used in conjunction with AO/PI fluorescence viability dyes provides easy, rapid, and accurate sample analysis. Learn more about counting isolated nuclei on the CellDrop.

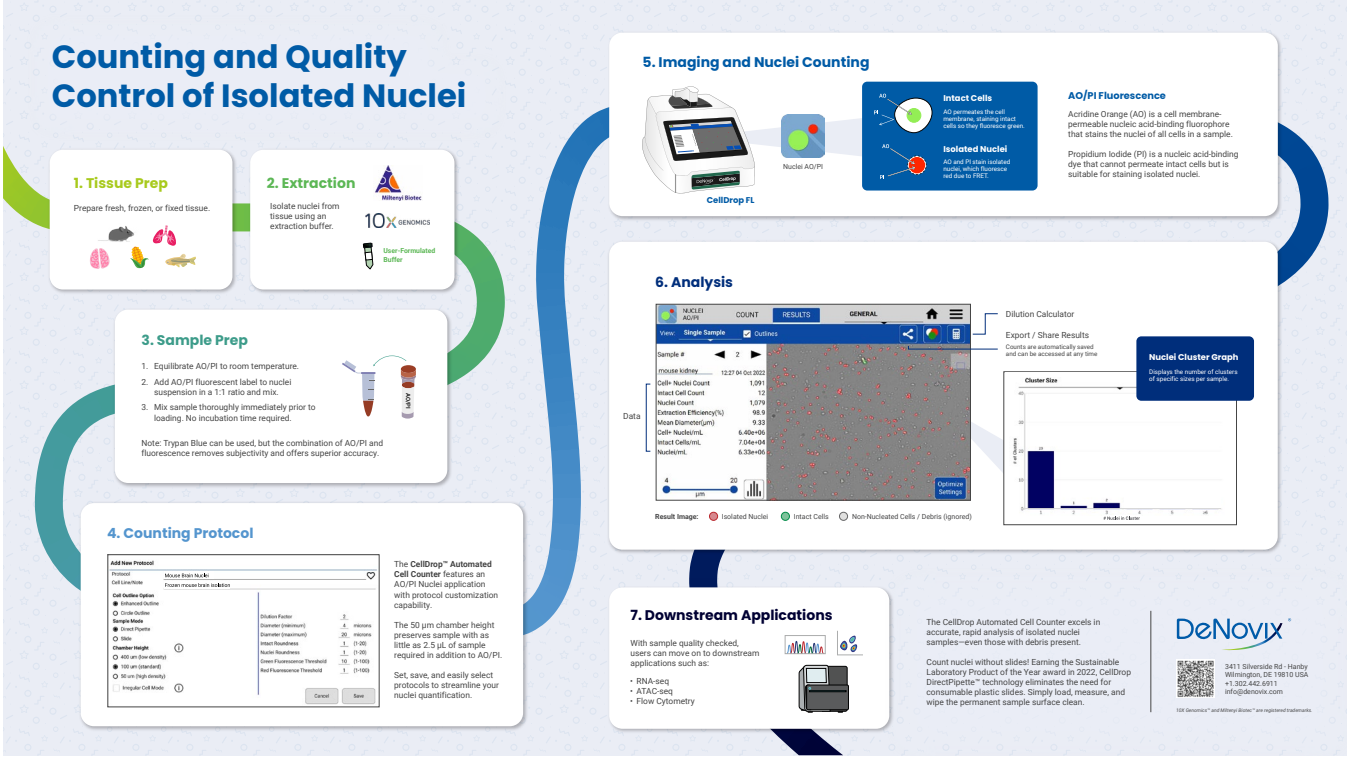
INFOGRAPHIC

Counting & Quality Control of Isolated Nuclei

As isolated nuclei samples are prepared for single-cell sequencing workflows, it's important to perform quality control checks. The CellDrop Automated Cell Counter offers a quick and reliable method for quantifying nuclei, measuring extraction efficiency, and visualizing debris in a sample.

Download our infographic to explore recommended methods for optimizing nuclei counts before downstream sequencing applications.

View Now



TECH TEAM TIPS

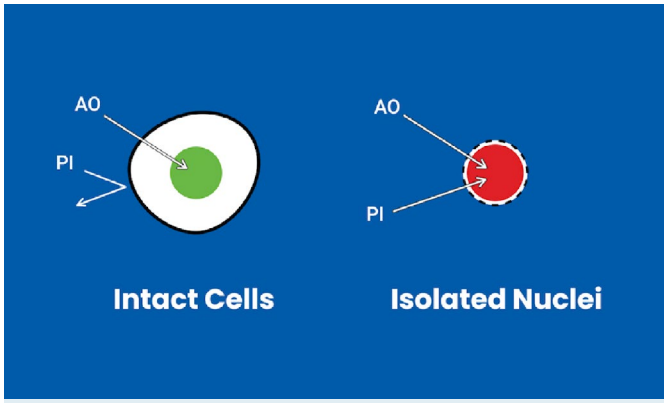
Nuclei Isolation Workflow

Watch this 5 minute Tech Team Tips video for a walk-through of counting nuclei on the CellDrop Automated Cell Counter.

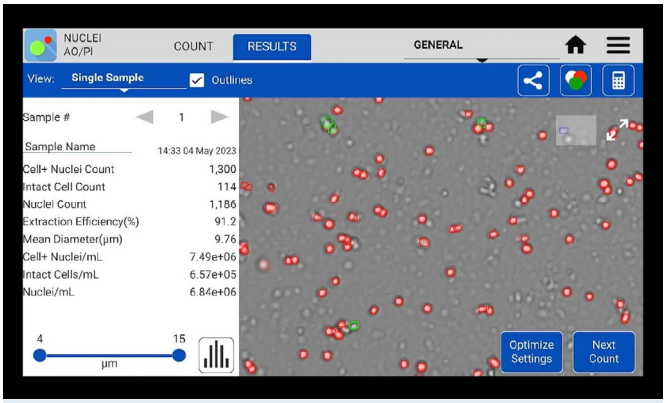
Watch Now

Ben demonstrates an isolated nuclei extraction, count, and analysis

Top tips for ensuring high yields in nuclei extractions



Learn how the fluorescence stain AO/PI effectively analyzes nuclei suspensions



Explore how results are reported and analyzed on the CellDrop's Nuclei AO/PI app

BENCH TIPS

Best Practices for Nuclei Extraction and Counting in Single-Cell Sequencing

ORIGINALLY PUBLISHED ON Biocompare
The Buyer's Guide for Life Scientists

Single-cell sequencing is a powerful tool for studying cellular heterogeneity, and using isolated nuclei for this process offers several advantages such as minimized dissociation bias, suitability with frozen or FFPE samples, and avoidance of stress responses from dissociation.[1,2] The success of this methodology is dependent on efficient isolations and accurate counting of nuclei. This article provides valuable insights and recommendations for optimizing nuclei extraction and counting, enabling important downstream applications including single-nucleus RNA sequencing (snRNA-seq) and assay for transposase-accessible chromatin sequencing (snATAC-seq).

Nuclei Extractions

After preparing the desired tissue—whether it's fresh, frozen, or fixed—the next step is the critical task of isolating nuclei. The isolation process requires a specific extraction buffer designed to separate the nuclei without disrupting them. There are several commercially available buffers, but some researchers opt for a custom-formulated buffer. Given the fragility of nuclei and their vulnerability to osmotic lysis, there are several techniques that can enhance the extraction process.

Tip 1: Prepare All Materials in Advance

For a smoother extraction workflow, have all the materials needed for the protocol set up before starting. This includes preparing the necessary buffers and ensuring the instruments are set according to the protocols. Additionally, buffers should be adjusted to the appropriate temperature to eliminate unnecessary waiting periods.

Tip 2: Maintain Cold Samples

Keep the samples cold both during and after the extraction. This simple precaution prevents nuclei lysis and can increase the efficiency of the experiment.

Tip 3: Time is of the Essence

Work quickly and avoid any pauses in the protocol. Delays can adversely affect the results of the extraction. As previously emphasized, pre-arranging the necessary materials allows for a faster and more efficient workflow.

Tip 4: Identify Proper Clean-Ups

The extraction process varies across tissue types, and depending on the tissue, different pore sizes may be required during the isolation step. Select a cleanup method appropriate for the tissue type in order to remove as much cellular debris as possible; the carryover of this debris can cause complications in downstream stages.

Tip 5: Perform a Trial Run

Since sequencing experiments can be costly and time consuming, if possible, it is good practice to perform a trial run on your samples and reagents if you are using a new method for the first time. Isolated nuclei can be QC tested using an automated cell counter with fluorescence counting capabilities.

Imaging and Nuclei Counting

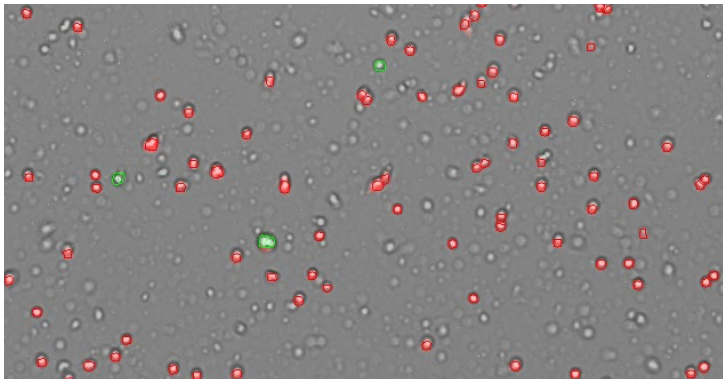
Nuclei counting and viability assays typically involve Trypan Blue or fluorescence-based staining techniques.

Trypan Blue facilitates the counting process by selectively coloring cells with compromised membranes (including the nuclei), giving them a distinct darkened appearance. Although traditionally considered a staple method, Trypan Blue staining has its drawbacks. Over time, Trypan Blue can crystallize, introducing debris that complicates the counting process. Cellular debris may also take on a dark appearance, making it challenging for automated and manual counting methods to distinguish between debris and dead cells.

Fluorescence-based assays, particularly Acridine Orange / Propidium Iodide (AO/PI), are the recommended choice as they provide a more objective and accurate count. While AO enters live and unlysed cells, emitting a green fluorescence, PI permeates dead or compromised cells and produces a red signal that labels isolated nuclei. This method avoids any crosstalk as the PI signal absorbs the AO signal due to Förster resonance energy transfer (FRET). Furthermore, each dye only becomes fluorescent upon binding to nucleic acid, which prevents background fluorescence and ignores cellular debris to ensure a more accurate nuclei count than Trypan Blue techniques.

Both counting processes are further improved through the use of automated cell counters. They enhance the precision and consistency of cell counting over manual methods by eliminating user bias and adopting standardized procedures.

Key features of these instruments include low volume requirements for analysis and slide-free operation, which conserves scarce samples and supports environmentally friendly practices. Automated systems also speed up the workflow and allow users to efficiently process a higher throughput of samples to provide more data for robust analysis.



Caption: Result image of mouse kidney nuclei. Isolated nuclei appear red, intact cells green, and debris remains unstained. Captured on DeNovix CellDrop Automated Cell Counter.

Preparation Steps for Counting QC

Once the nuclei are successfully isolated, they need to be counted and quality-checked before proceeding to the library preparations. Precise counting begins with the preparation of the reagents AO and PI, collectively referred to as AO/PI.

Tip 1: Equilibrate AO/PI to Room Temperature

One of the foremost steps in this process is to ensure that the AO/PI solution is equilibrated to room temperature before use. Room temperature AO/PI solution will exhibit the highest fluorescence yield, ensuring optimum efficiency of the assay.

Tip 2: Optimize Nuclei Counting Volume

The counting process involves staining and counting a subset of the isolated nuclei, which, once stained, are unsuitable for the downstream experiment. Due to the small quantity of samples obtained from these extraction methods, conserving nuclei by using only what is necessary for quantification is essential. Fortunately, advanced counting systems allow for the use of minimal input volumes. This allows users to preserve the majority of the sample for the intended experimental procedures.

Tip 3: Gently Resuspend Delicate Nuclei

After adding the staining solution, homogenously resuspended the nuclei before loading. However, rigorous vortexing and pipetting will disrupt the nuclei and lead to sample loss. Considering their fragility, either light pipetting or gentle mixing is the best option for resuspension.

Tip 4: Minimize Delay after Mixing

Reduce interruptions in the protocol by continuing to move quickly after the nuclei are combined with AO/PI. Prolonged exposure or delays can adversely affect the nuclei, leading to increased osmotic lysis and lower efficiency of the downstream workflow.

Caption: Once nuclei suspension is thoroughly mixed with AO/PI, the sample can undergo a QC check on an automated cell counter.

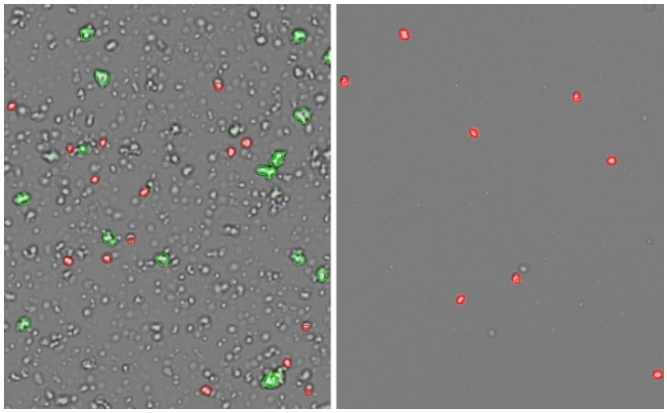
Cleaning Debris from Samples

The presence of debris during cell counting can vary based on the extraction process and the nature of the sample. Although sophisticated cell counting technologies are capable of accurate identification in the presence of debris, minimizing this debris is better for downstream applications.

Carryover debris from extractions poses several challenges. Not only can it obstruct microfluidic systems, but it can also occupy wells intended for nuclei, leading to wasted resources. In addition, difficult samples like those from the brain and heart require thorough nuclei purification because they inherently contain more debris compared to other sample types.

In instances where significant cellular debris is detected during the counting stage, the best course of action is to utilize nuclei clean-ups.

A popular approach involves kits equipped with magnetic microbeads. These microbeads bind to the nuclei and facilitate the removal of debris and live cells, which efficiently purifies samples without compromising yields.



Caption: Left–Isolated nuclei from mouse brain tissue. Right–Same sample after purification with Anti-Nucleus Microbeads (Miltenyi Biotec).

CellDrop Automated Cell Counter

Application Area: Isolating nuclei for epigenetic studies

Institute: Sanford Burnham Prebys Medical Discovery

"Easy to use and robust results."

★★★★★

"I love this counter, it is extremely easy to use, fast and robust. Honestly, the counter is a bit more expensive than others in the market but it is so reliable and fast that it is worth every penny."

Conclusion

Accurate nuclei isolation, counting and QC are vital for reliable downstream applications like single-cell sequencing. Following these best practices enables scientists to refine their nuclei protocols, leading to improved outcomes and more meaningful scientific discoveries.

For scientists aiming to optimize their single-cell genomics workflows, the CellDrop Automated Cell Counter is an excellent choice and eliminates the need for disposable plastic slides. DeNovix CellDrop instruments are standalone with intuitive software that provides dedicated applications for Isolated Nuclei QC, primary cells and cell cultures. CellDrop simplifies the process of automatic cell counting and viability assessments, ensuring efficient and accurate results. To learn more about the CellDrop and how it can benefit your single-cell research, visit denovix.com or see how it is used in the nuclei counting workflow in this webinar.

1. Habib, N., Avraham-Davidi, I., Basu, A., Burks, T., Shekhar, K., Hofree, M., Choudhury, S. R., Aguet, F., Gelfand, E., Ardlie, K., Weitz, D. A., Rozenblatt-Rosen, O., Zhang, F., & Regev, A. (2017). Massively parallel single-nucleus RNA- seq with DroNc-seq. Nature methods, 14(10), 955–958. https://doi.org/10.1038/nmeth.4407

2. Gaublomme, J. T., Li, B., McCabe, C., Knecht, A., Yang, Y., Drokhlyansky, E., Van Wittenberghe, N., Waldman, J., Dionne, D., Nguyen, L., De Jager, P. L., Yeung, B., Zhao, X., Habib, N., Rozenblatt-Rosen, O., & Regev, A. (2019). Nuclei multiplexing with barcoded antibodies for single-nucleus genomics. Nature communications, 10(1), 2907. https://doi.org/10.1038/s41467-019-10756-2

WEBINAR HIGHLIGHT

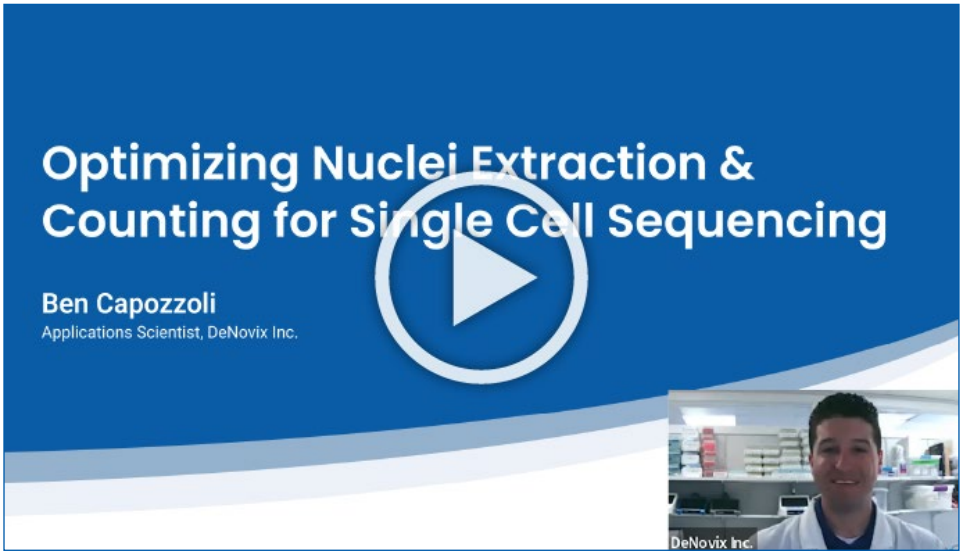
Optimizing Nuclei Extraction & Counting for Single-Cell Sequencing

In this webinar, DeNovix Application Scientist Ben Capozzoli discusses methods and tips for preparing nuclei samples before single-cell sequencing workflows.

Our featured method for achieving accurate, reproducible nuclei counts is pairing the CellDrop Automated Cell Counter with AO/PI fluorescent label. Ben performs a short demonstration of an isolated nuclei count using the CellDrop's dedicated Nuclei AO/PI app.

- Tips for optimizing nuclei extraction protocols
- Real-time nuclei counts on the CellDrop Automated Cell Counter
- Q&A session with DeNovix scientists

Watch Now On Demand



View Webinar

QUESTIONS AND ANSWERS

Q&A: Optimizing Nuclei Extraction and Counting

During our Optimizing Nuclei Extraction & Counting for Single-Cell Sequencing webinar, we conducted a live question and answer session with our applications team. This article contains some of the top questions answered by DeNovix scientists during the webinar.

What is the minimum amount of nuclei sample you recommend using on the CellDrop?

The minimum amount of sample we would recommend using for the 50 µm chamber height is 2.5 µL of your nuclei solution & 2.5 µL of your AO/PI. Mix them together for a total of 5 µL to load into the 50 µm height. In practice it would be a little better to account for pipetting error and probably do 3 µL and 3 µL. However, you can get away with as little as 2.5 and 2.5 if you're really careful about your pipetting.

How does the CellDrop handle irregularly shaped nuclei?

The CellDrop and a lot of other automated cell counters are designed to count circles. Most tissue culture cells and PBMCs are circular, but we have a modification in our algorithm called "Irregular Cell Mode" that you can toggle on to better process different shapes. There's a technical note on our website about how it works, but in short, you can turn that on if your sample contains non-circular cells. After you check the box to turn on irregular cell mode, it allows our algorithm to better process oval or oblong shaped nuclei, which are common in certain samples e.g. those that contain smooth muscle. Smooth muscle nuclei are known to be almost rod shaped in how they appear. The heart is a good example where we see a lot of these.

Specifically for nuclei, could you just use AO or PI, or do you need to use both?

You could use just one or the other, but you won't get all the same information as the combination stain. If you use just AO, you wouldn't have any idea of extraction efficiency. It would stain your nuclei, but it would also stain your live cells. You wouldn't know which objects are live, intact cells that weren't lysed by the extraction, or which are your isolated nuclei. You would hope that they're mostly nuclei, but you wouldn't have any idea if your extraction really went well.

Similarly, if you just PI, it'll do fine staining just nuclei, but you won't have a super strong idea of how many live cells are in your mixture. Knowing how much debris and how many other particles of interest are present is critical for a high quality library prep. You want to make sure that there's nothing taking up space on the microfluidic when you go to process for the single-cell sequencing. These are also very low cost assays, so there isn't a tremendous amount of benefit in just using one of the fluorophores.

Using the CellDrop, how do the results compare to using a hemocytometer or a flow cytometer?

There's a lot of subjectivity with counting on a hemocytometer. If you did a large study, say 10 replicates, the CellDrop tends to align closely with the average answer. But the precision of the CellDrop is much tighter than the hemocytometer. With hand counts, you'll see a large variation from count-to-count and user-to-user, as well as users having to do so much interpretation of the image. CellDrop standardizes the count settings, which removes subjectivity. This is especially the case when using fluorescence, where the presence of red or green fluorophores remove the subjectivity associated with Trypan Blue.

In terms of a flow cytometer, we have some data that shows we are very closely aligned with the flow cytometer we've worked with. We're in the process of developing a technical note that displays that data comparison, but there was no statistical difference between the counts on the CellDrop versus the flow cytometer that we worked with. So we do compare favorably with both technologies, just a little differently.

Can you differentiate nuclei from dead cells?

You cannot by using AO/PI, but you can via size. Dead cells are a lot larger than an extracted nucleus. However, it's highly unlikely that a dead cell would make it all the way through the extraction process without lysing the nucleus out. There are so many washing, filtration, and spinning steps that the chances of a large population of dead cells making it through is statistically low. But again, you could gate via size in the red channel to eliminate any really large dead objects. That would eliminate any small number of dead cells that make it through.

What are the concentration ranges for CellDrop?

So the reason we have different chamber heights is to accommodate a really wide variety of concentrations or cell / nuclei densities. The 50 µm height is best for really dense or concentrated samples. For the most part we recommend using that upwards of 10 million cells per mil. Anything under that, and down to about a hundred thousand cells per mil, we recommend the 100 µm height. From 100,000 cells per mil to about 700 cells per mil, we recommend the 400 µm chamber height. That one is four times as high as your standard hemocytometer, so instead of 10 µL, you would load 40 µL. That might be less than ideal for a nuclei sample, but those numbers are based on statistical significance.

Which debris removal protocol / method do you use for nuclei prep?

Typically, selecting the proper strainers will greatly reduce the level of debris. We have the most experience with the Miltenyi system. There is also an option to further purify the nuclei from any residual debris using the Miltenyi nuclei clean up kit.

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