Dec 15, 2020

Quantitative Assessment of Islet Viability upon Arrival to HIPP by Staining with Trypan Blue Dye

IIDP-HIPP [1]

[1]Integrated Islet Distribution Program and Human Islet Phenotyping Program

1 Works for me dx.doi.org/10.17504/protocols.io.bp97mr9n

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IIDP-HIPP

ABSTRACT

This Standard Operating Procedure (SOP) is based on the Vanderbilt Human Islet Phenotyping Program (HIPP) procedures for dispersing islets to single cell suspension and quantitatively assessing viability by Trypan Blue staining.

This SOP defines the assay method used by the Human Islet Phenotyping Program (HIPP) for quantitative and qualitative determination of the Purified Human Pancreatic Islet product, post-shipment, manufactured for use in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored research in the Integrated Islet Distribution Program (IIDP).

This Standard Operating Procedure (SOP) #: HIPP-04-v02

DOI

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KEYWORDS

HIPP, purified human pancreatic islet, staining with trypan blue dye, islet viability

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GUIDELINES

1. *Integrated Islet Distribution Program (IIDP) (RRID:SCR_014387):* The IIDP is a grant funded program commissioned and funded by the NIDDK to provide quality human islets to the diabetes research community to advance scientific discoveries and translational medicine. The IIDP consists of the NIDDK Project Scientist and Program Official, the External Scientific Panel and the CC at City of Hope (COH). The IIDP CC integrates an interactive group of academic laboratories including the subcontracted IIDP centers.

2. *IIDP Coordinating Center (CC):* Joyce Niland, Ph.D. and Carmella Evans-Molina, M.D., Ph.D. serve as Co-Principal Investigators (Co-PIs) for the IIDP Program located within the Department of Diabetes and Cancer Discovery Science at COH to coordinate the activities of the IIDP and Human Islet Phenotyping Program (HIPP). Dr. Niland, contact PI, oversees the daily activity of the IIDP staff, provides informatics/ biostatistical input, and subcontracts with the Islet Isolation Centers (IICs) to ensure the delivery of the highest quality human islets to IIDP-approved investigators. Dr. Evans-Molina serves as the liaison to the HIPP, interacting closely to ensure that extensive, high quality phenotypic data are collected on islets distributed by the IICs. She also facilitates the delivery of this information to both the IICs and the IIDP-approved investigators, while responding to questions, issues, or suggestions for further HIPP enhancements.

3. *Human Islet Phenotyping Program (HIPP):* The HIPP is a subcontracted entity of the IIDP through the COH and Vanderbilt University. The HIPP is directed by Marcela Brissova, Ph.D. and is responsible for performing specific standardized quality control assays agreed upon by both the IIDP and the HIPP, in order to provide enhanced, quality data on the human islets post-shipment, to the IIDP. The results of these assays will be approved by the CC and posted on the IIDP website for both the centers and the approved investigators.

4. *Islet Equivalent (IEQ):* An islet with a diameter of 150 µm determined mathematically by compensating for islet shape.

5. *Trypan Blue stain:* A vital stain used to selectively color dead tissues or cells blue. Trypan Blue is a diazo dye. In a viable cell Trypan Blue is excluded, however, it traverses the membrane in a dead cell (similar to propidium iodide). Hence, dead cells have a distinctive blue color under a microscope.

References:

Louis KS, Siegel AC. Cell Viability Analysis Using Trypan Blue: Manual and Automated Methods. Mammalian Cell Viability, Totowa, NJ: Humana Press; 2011. p. 7–12. https://pubmed.ncbi.nlm.nih.gov/21468962/

Bansal H, Yihua Q, Iyer SP, Ganapathy S, Proia DA, Proia D, et al. WTAP is a novel oncogenic protein in acute myeloid leukemia. Leukemia. 2014 May;28(5):1171–4. PMCID: PMC4369791. https://www.nature.com/articles/leu201416

MATERIALS TEXT

Equipment:

1. Centrifuge (Eppendorf 5702R)

2. Countess II (Life Technologies AMQAX1000)

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3. Eppendorf Xplorer electronic pipette (Eppendorf 2231000727) or equivalent

Supplies and Materials:

1. 1X Phosphate buffered saline without Ca/Mg (PBS, Invitrogen 14190-144)

2. 0.5 M Ethylenediaminetetraacetic acid, pH **8.0** (EDTA, Cellgro 46-034-CI)

3. Accutase (Innovative CellT AT-104)

4. CMRL1066 (Cellgro 15-110-CV)

5. L-Glutamine (Invitrogen 25030-081)

6. Penicillin/Streptomycin (Invitrogen 15140-122)

7. Fetal Bovine Serum (FBS Millipore TMS-013-B)

8. Trypan Blue (Invitrogen T10282)

9. Chamber slide (Invitrogen C10283)

10. 1.5mL Eppendorf tubes (Denville C-2171)

Centrifuge
5702R
Eppendorf **None**

Countess II
AMQAX1000
Life Technologies/Invitrogen **None**

PDF

Eppendorf Xplorer electronic pipette
Eppendorf 2231000727 or equivalent
Eppendorf **None**

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1X Phosphate buffered saline without Ca/Mg **Thermo Fisher Scientific Catalog #Invitrogen 14190-144**

0.5 M Ethylenediaminetetraacetic acid pH8.0 (EDTA Cellgro 46-034-Cl) **VWR Scientific Catalog #46-034-Cl**

Accutase (cell detachment solution) **Innovative Cell Technologies, Inc Catalog #AT-104**

CMRL1066 **Corning Catalog #15-110-CV**

L-Glutamine **Invitrogen - Thermo Fisher Catalog #25030-081**

Penicillin/Streptomycin **Invitrogen - Thermo Fisher Catalog #15140-122**

Fetal Bovine Serum **Emd Millipore Catalog # TMS-013-B**

Trypan Blue **Invitrogen - Thermo Fisher Catalog #T10282**

Chamber slide **Invitrogen - Thermo Fisher Catalog #C10283**

1.5mL Eppendorf tubes **USA Scientific Catalog #Denville C-2171**

SAFETY WARNINGS

EDTA (0.5M Ethylenediaminetetraacetic acid, pH **8.0** **)**

Wear eye/face protection. Do not breathe dust/fume/gas/mist/vapors/spray.

EDTA .5 PH=8 1LT.pdf

Trypan Blue stain

May cause cancer.
Suspected of damaging fertility or the unborn child if inhaled
Obtain special instructions before use
Do not handle until all safety precautions have been read and understood.
Wear protective gloves/protective clothing/eye protection/face protection

Trypan blue10282_MTR-NALT_EN.pdf

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ABSTRACT

This Standard Operating Procedure (SOP) is based on the Vanderbilt Human Islet Phenotyping Program (HIPP) procedures for dispersing islets to single cell suspension and quantitatively assessing viability by Trypan Blue staining.

This SOP defines the assay method used by the Human Islet Phenotyping Program (HIPP) for quantitative and qualitative determination of the Purified Human Pancreatic Islet product, post-shipment, manufactured for use in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored research in the Integrated Islet Distribution Program (IIDP).

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Procedures

1 Dispersion of islets into single cell suspension

1.1 Prepare 2 aliquots of approximately 100 handpicked islets in **1.5 mL** tubes to determine viability of islets. ***Note:*** This is important especially for islet preparations with lower purity where cell death in the contaminating exocrine compartment may occur at higher rate compared to islets and thus could lead to underestimating islet viability.

1.2 Wash islets with 2mM EDTA made in 1X PBS and centrifuge them at 200 rcf for 1 minute at **4 °C** . Repeat two times.

1.3 After last wash, resuspend the pellet in **500 µl** of Accutase.

1.4 Triturate islets at slow speed for 10 minutes at **37 °C** using an electronic multichannel pipet with **200 µl** tips. ***Note:*** As islets are getting dispersed, the sample should become slightly opaque.

1.5 Quench the reaction with **500 µl** of CMRL-1066 media containing 10% FBS, 2mM L-glutamine, 1% penicillin/streptomycin.

1.6 Rinse the cells with **1 mL** of CMRL-1066 medium, centrifuge at 500 rcf for 3 minutes at **4 °C** . Carefully remove the media without disturbing the pellet at the bottom of the tube. Repeat one more time.

1.7 Resuspend the cell pellet in 50-75 µL of CMRL-1066 medium by gently flicking the tube and keep the sample on ice. The volume of CMRL used is determined by the relative size of the cell pellet.

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2 Cell counting

2.1 From each islet sample, transfer two **10 µl** aliquots of the cell suspension into **1.5 mL** tubes. Mix well before pipetting the sample.

2.2 Prepare the cell counting chamber slide for sample loading (slide has two chambers and looks similar to the hemocytometer but without grids).

2.3 Centrifuge Trypan Blue stock at 3,000 rcf for 1 minute to pellet particulates that may be present in the dye.

2.4 Gently mix the first aliquot of the islet cell suspension with **10 µl** of Trypan Blue dye and then load **10 µl** of the mixture into the chamber slide.

2.5 Wait approximately 20 seconds for the cells to spread out on the slide, insert the slide into the Countess II cell counter and acquire cell counts following instrument guidelines. The output of cell and viability counts for the cell suspension of purified islets is illustrated in **Figure 1**.

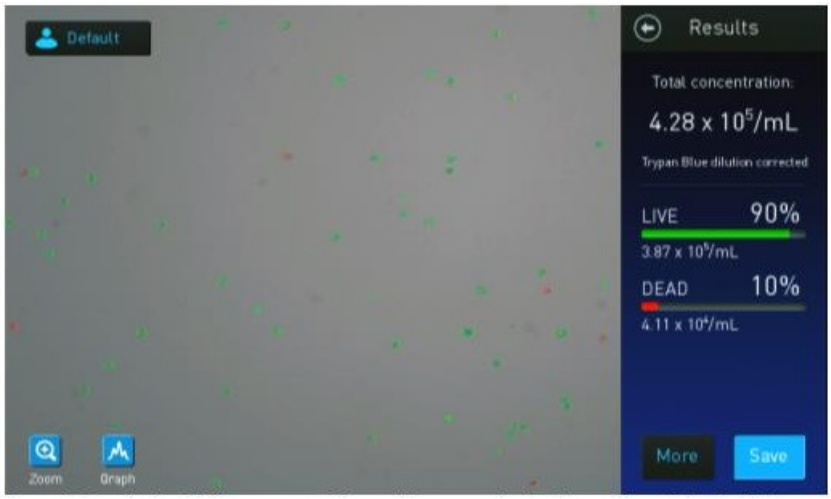

Figure 1. Quantitative viability assessment of human islet preparations by Trypan Blue staining. Purified human islets were dispersed with Accutase. Cell suspension was labeled with Trypan Blue and viability measured using Countess II automated cell counter. The output image of the cell counter on the left shows viable cells in ***green*** and dead cells in ***red***. The total cell concentration and viability are reported in the panel on the right-hand side.

2.6 Repeat cell counts for the second aliquot of the same islet sample using the second slide chamber.

2.7 Repeat steps 2.2 through 2.5 for the remaining sample.

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2.8 **Note:** Cells need to be imaged within 15 minutes of Trypan Blue addition due to cytotoxicity of the dye.

2.9 Report average values of viable cells for duplicates of purified islets based on two cell counts per each replicate.

Data Storage and Reporting

3 Offload the data, including cell images, from the cell counter using a USB drive and transfer to the Vanderbilt HIPP server-based platform for storage and analysis.

4 Islet viability data reporting will be uploaded within 1 business day to the IIDP-HIPP database and immediately disseminated to IIDP-affiliated investigators and islet isolation centers.

Deviations and Resolutions

5 Document any deviations that occurred during this protocol that affect the final results and report with the analysis of the assay.

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