View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Liverpool Repository


Bio-protocol 9(xx): exxxx.
DOI:10.21769/BioProtoc.xxxx

# Pancreatic Acinar Cell Preparation for Oxygen Consumption and Lactate Production Analysis

Jane A Armstrong[1], Robert Sutton[1] and David N Criddle[2]

[1]Department of Clinical Cancer Medicine, Institute of Translational Medicine, University of Liverpool, UK; [2]Department of Molecular and Cellular Physiology, Institute of Translational Medicine, University of Liverpool, UK

*For correspondence: criddle@liv.ac.uk

**[Abstract]** Mitochondrial dysfunction is a principal feature of acute pancreatitis (AP) although the underlying mechanisms are still unclear. AP precipitants induce $Ca^{2+}$-dependent formation of the mitochondrial permeability transition pore (MPTP) in pancreatic acinar cells (PACs), leading to ATP depletion and necrosis. Evaluations of mitochondrial bioenergetics have mainly been performed in isolated PACs using confocal microscopy, with assessment of mitochondrial membrane potential, NADH/$FAD^+$ and ATP levels, coupled with patch-clamp electrophysiology. These studies are technically demanding and time-consuming. Application of Seahorse flux analysis now allows detailed investigations of bioenergetics changes to be performed in cell populations using a multi-well plate-reader format; rates of oxygen consumption (OCR) and extracellular acidification (ECAR) provide important information about cellular respiration and glycolysis, respectively. Parameters such as maximal respiration, ATP-linked capacity and proton leak can be derived from application of a respiratory function "stress" test that involves pharmacological manipulation of the electron transport chain. The use of Seahorse Flux analysis therefore provides a quick, and convenient means to measure detailed cellular bioenergetics and allows results to be coupled with other plate-reader based assays, providing a fuller understanding of the pathophysiological consequences of mitochondrial bioenergetics alterations.

**Keywords:** Mitochondrial dysfunction, Bioenergetics, Seahorse, Respiration, Glycolysis, Pancreatic acinar cells, Acute pancreatitis

**[Background]** Mitochondrial dysfunction is a core feature of acute pancreatitis (AP), a debilitating and potentially fatal disease for which there is currently no specific therapy (Criddle 2016; Habtezion *et al.*, 2019). The elucidation of pivotal pathological mechanisms which underlie mitochondrial damage in pancreatic acinar cells (PACs) is paramount for the development of new therapies. Previously evaluation of mitochondrial bioenergetics in isolated PACs have been mostly performed using confocal microscopy, including assessments of mitochondrial membrane potential (tetramethyl rhodamine methyl ester: TMRM), NADH/$FAD^+$ autofluorescence and ATP (Magnesium Green), coupled with patch-clamp electrophysiology (Voronina *et al.*, 2002; Criddle, *et al.,* 2004; Criddle *et al.*, 2006). Such studies have pinpointed a reduction of ATP in response to precipitants of AP, via the opening of the mitochondrial permeability transition pore (MPTP), as a critical event that leads to necrotic cell death (Criddle *et al.*, 2006; Mukherjee *et al.*, 2016); supplementation with intracellular ATP ameliorated damage. Furthermore,

40 luciferase measurements in PACs have provided details of changes of both mitochondrial and cytosolic
41 ATP concentrations induced by pathophysiological stimulation (Voronina *et al.*, 2010). Such
42 experimental approaches in single cells, however, are technically difficult and time-consuming. In
43 contrast, population-based assays provide important information about mitochondrial dysfunction and
44 cell death using a convenient plate-reader format (Armstrong *et al.*, 2019). The use of Seahorse Flux
45 analysis allows a detailed evaluation of bioenergetics changes to be performed in PACs, measuring
46 rates of oxygen consumption (OCR) and extracellular acidification (ECAR); these inform about cellular
47 respiration and glycolysis, respectively. A respiratory function "stress" test can further be applied in which
48 pharmacological manipulation of the electron transport chain (ETC) is used to derive parameters such
49 as the maximal respiration, Spare Respiratory Capacity, ATP-linked turnover and non-mitochondrial
50 respiration. Such detailed bioenergetics information can be coupled with parallel studies of apoptosis
51 and necrosis to inform the influence of mitochondrial dysfunction on cell death patterns (Armstrong *et*
52 *al.*, 2018).
53
54 **Materials and Reagents**
55
56 1. 70 µm filters (Fisherbrand, catalog number: 22363548)
57 2. Tissue paper
58 3. 30G needle
59 4. 1.5 ml microcentrifuge tube
60 5. 15 ml falcon tube
61 6. Pipette tips
62 7. Male CD1 or C57BL6/J mice, 8-12 week old (Charles River).
63 8. Collagenase (Worthington Biochemical Corporation, Lakewood, NJ)
64 9. PI-Cassette$^{TM}$ (Chemometec, Nucleocounter, catalog number: 941-0001)
65 10. Reagent A100, Lysis buffer (SKU: 910-0003, Chemometec, Nucleocounter)
66 11. Reagent B; Stabilizing buffer (SKU: 910-0002, Chemometec, Denmark)
67 12. XF24 Fluxpak, containing cell plates, cartridge consisting of sensor and utility plates and
68 Seahorse XF calibrant (Agilent, Seahorse, catalog number: 100850-100)
69 13. Matrigel basement membrane matrix (Corning, catalog number: 354234)
70 14. Dimethyl sulfoxide (DMSO, Sigma, catalog number: D2650)
71 15. Ethyl alcohol, Pure (Sigma, catalog number: 459836)
72 16. Oligomycin A (Sigma, Merck, catalog number: 75351)
73 17. Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP, C2920, Sigma, Merck)
74 18. Antimycin A from *Streptomyces* sp. (Sigma, Merck, catalog number: A8674)
75 19. Rotenone (Sigma, Merck, catalog number: R8875)
76 20. DMEM (Sigma, Merck, catalog number: D5030)
77 21. L-Glutamine 100x (Gibco, Life Technologies, catalog number: 25030-081)
78 22. D-Glucose (Sigma, Merck, catalog number: G7528)

79 23. Sodium pyruvate (Sigma, Merck, catalog number: P8574)
80 24. Sodium chloride (Sigma, Merck, catalog number: S9888)
81 25. Potassium chloride (Sigma, Merck, catalog number: P3911)
82 26. Magnesium chloride (Sigma, Merck, catalog number: M1028)
83 27. Calcium chloride (Sigma, Merck, catalog number: 21115)
84 28. Extracellular solution (see Recipes)
85 29. Seahorse media (see Recipes)
86
87 **Equipment**
88
89 1. Surgical scissors
90 2. Water bath
91 3. -20 °C freezer
92 4. Seahorse XF24 Extracellular Flux Analyser (Agilent)
93 5. Cell Counter NucleoCounter® NC-100™ (900-0004, ChemoMetec, Denmark)
94 6. Inverted light microscope
95
96 **Software**
97
98 1. Wave (Seahorse, Agilent. https://www.agilent.com)
99 2. Prism (GraphPad Software Inc., La Jolla, CA. https://www.graphpad.com)
100
101 **Procedure**
102
103 **Day 1**
104 A. *Pancreatic acinar cell preparation*
105 1. Terminate young (8-12 week old) adult CD1 or C57BL/6 (wild type) mouse using an approved
106 Schedule 1 procedure.
107 2. Place mouse down on right hand side on fresh tissue paper.
108 3. Using forceps and scissors cut away fur in the side abdominal area. Once removed make a
109 similar removal of lower skin epidermis to expose the abdominal cavity.
110 4. Locate the pancreas below the spleen gently with forceps and cut free with a small pair of
111 surgical scissors.
112 5. Place the freshly excised pancreas in 7 ml extracellular solution and keep on ice until digestion.
113 6. Pre-warm 1 ml of collagenase (200 U $ml^{-1}$) to 37 °C in a water bath.
114 7. Carefully inject the pre-warmed collagenase using a 30G needle into the pancreas samples
115 placed in a weighing boat until the pancreas inflates. Draw up any remaining collagenase not
116 retained within the pancreas from the weighing boat and re-inject into pancreas.
117 8. Place the inflated pancreas into a 1.5 ml microcentrifuge tube and add any remaining

118 collagenase.
119 9. Place in a 37 °C water bath for 17 min
120 *Note: This time is dependent on the brand and concentration of collagenase used. You may*
121 *need to optimize so that you get a optimal amount of cell viability and cell number achieved.*
122 10. After incubation for 17 min remove from water bath and decant contents of the 1.5 ml
123 microcentrifuge tube into a 15 ml falcon tube labelled 'Panc', quickly add 5 ml of extracellular
124 solution.
125 11. Using a 1 ml pipette tip, cut at an angle across the tip and the volume set at 700 µl vigorously
126 pipette the whole pancreas tissue up and down in and out of the pipette tip to allow for tissue
127 dissociation (trituration).
128 12. Repeat pipetting up and down until the mixture becomes cloudy with the release of cells.
129 13. Upon cessation of pipetting quickly remove the cellular solution into a fresh 15 ml tube labelled
130 'Cells', using a squeezy pipette being careful to leave behind any pieces of pancreas tissue.
131 14. Add 5 ml of extracellular solution to the 15 ml tube 'Panc'.
132 15. Using a fresh 1 ml pipette tip, cut at smaller angle than previously across the tip and the volume
133 set at 700 µl vigorously pipette the whole pancreas tissue in and out of the pipette tip to allow
134 further tissue dissociation.
135 16. Upon cessation of pipetting quickly remove the cellular solution into 15 ml tube 'Cells', using a
136 squeezy pipette and being careful to leave behind any pieces of pancreas tissue.
137 17. Repeat Steps A14-A16 for a third and final time.
138 18. Discard any tissue remaining in a 15 ml tube labelled 'Panc'
139 19. Centrifuge 15 ml tube labelled 'Cells' for 2 min, 130 *x g*, room temp.
140 20. Carefully pour off the supernatant leaving behind the loose cell pellet.
141 21. Resuspend the cell pellet in 1 ml of extracellular solution.
142 22. Add an additional 9 ml of extracellular solution.
143 23. Set up a 70 micron filter in a 50 ml tube (labelled and dated) and pre-wet with a small amount
144 of extracellular fluid.
145 24. Filter the 10 ml of cell suspension from the 15ml tube 'Cells' through the filter into the 50 ml tube.
146 25. Rinse the filter with plenty of extracellular fluid until the tube is filled to the 40 ml mark.
147 26. Centrifuge 50 ml tube labelled 'Cells' for 2 min, 130 *x g*, room temp.
148 27. Carefully pour off the supernatant leaving behind the loose cell pellet.
149 28. Resuspend the cell pellet in 1 ml of extracellular solution.
150 29. Add an additional 9 ml of extracellular solution.
151 30. Place the tube overnight in at 4 °C.
152
153 B. Oxygen consumption and lactate production analysis cell plate preparation and cartridge
154 rehydration
155 1. Remove a Seahorse XF 24 well cell plate and remove from packaging
156 2. Take a pre-aliquotted 250 µl vial of Matrigel from storage in a -80 °C freezer and thaw on ice.

157 3. Adapt Corning's 'Thin Coating Method' for use on a Seahorse XF24 cell plate as follows:
158 4. Using cooled pipettes, mix the BD Matrigel Basement Membrane Matrix to homogeneity.
159 5. Dilute Corning Matrigel Basement Membrane Matrix using serum-free medium Seahorse media.
160 6. Carefully add 36 µl diluted BD Matrigel Basement Membrane Matrix to Seahorse XF 24 well cell
161 plate being sure not to create any bubbles.
162 7. Incubate at room temperature for 30 min.
163 8. Aspirate unbound material and gently rinse using serum-free Seahorse media.
164 9. Pipette 100 µl serum-free Seahorse media into each well and leave at 4 °C until ready for use.
165 10. Remove a Seahorse XF 24 cartridge from packaging.
166 11. Remove the sensor plate (green upper half of the cartridge) and place lid down on the bench.
167 12. Pipette 1 ml of XF callibrant into each of the 24 wells of the utility plate and return the sensor
168 plate so the probes of each well are submerged in XF callibrant.
169 13. Place in a 37 °C $CO_2$ free incubator overnight until ready for use.
170
171 **Day 2**
172 C. Assess pancreatic cell viability
173 1. The next day the cell suspension is mixed to obtain a homogenous suspension by gently
174 pipetting up and down using a 1 ml pipette.
175 2. Pipette a 1 ml cell sample from the cell suspension into a 1.5 ml microcentrifuge tube.
176 3. Label a PI-Cassette™ 'NV' (non-viable).
177 4. Draw the cell suspension into a PI-Cassette™ by inserting the tip of the cassette into the cell
178 suspension in the 1.5 ml microcentrifuge tube and pressing the piston.
179 5. Set aside the 'NV' PI-Cassette™ to be analysed.
180 6. Pipette 100 µl of the 1ml cell sample from the 1.5 ml microcentrifuge tube into a second 1.5 ml
181 microcentrifuge tube. Return any cell sample remaining to the original cell suspension 50 ml
182 centrifuge tube 'Cells'.
183 7. Add 100 µl volume of Reagent A100 to the microcentrifuge tube with the 100 µl cell sample.
184 8. Mix by pipetting.
185 9. Add 100 µl volume of Reagent B to the mixture of cell suspension and Reagent A100.
186 10. Mix by pipetting.
187 11. Label a PI-Cassette™ 'T' (total)
188 12. Draw the diluted cell suspension into a PI-Cassette™ by inserting the tip of the cassette into the
189 cell suspension and pressing the piston.
190 13. Immediately place the loaded PI-Cassette™ in the NucleoCounter® NC-100™sample tray, press
191 RUN.
192 14. After approximately 45 s the total cell concentration (cells/ml) is presented in the bottom right.
193 15. The cell count produced will normally be in the order of x $10^5$. Divide by 10 to achieve x $10^6$ for
194 the following calculations
195 16. Calculate total cell number and percent viability as follows:

DOI:10.21769/BioProtoc.xxxx

196 Cell viability = [‘NV’ / (‘T’ x 3)] x 100
197 Total viable cell count = [(‘T’ x 3) – ‘NV’] x volume of cell suspension
198
199 Examples of total cell count and percent viability previously achieved when isolating acinar cells
200 from two separate pancreas (taken from two animals) at the same time and pooled for analysis
201 are shown in Table 1.
202
203 **Table 1. Examples of total cell count and percent viability previously achieved**

| Total read out | Total cell count | Non-viable cell count | Viability (%) | Volume (ml) | Total cell count (x10 $^{6}$) |
|---|---|---|---|---|---|
| 0.324 | 0.972 | 0.137 | 85.9 | 10 | 8.35 |
| 0.211 | 0.633 | 0.091 | 85.6 | 10 | 5.42 |
| 0.462 | 1.386 | 0.161 | 88.4 | 10 | 12.25 |
| 0.351 | 1.053 | 0.018 | 98.3 | 10 | 10.35 |
| 0.302 | 0.906 | 0.165 | 81.8 | 10 | 7.41 |
| 0.326 | 0.978 | 0.145 | 85.2 | 10 | 8.33 |
| 0.238 | 0.714 | 0.152 | 78.7 | 10 | 5.62 |
| 0.224 | 0.672 | 0.101 | 85.0 | 10 | 5.71 |
| 0.372 | 1.116 | 0.207 | 81.5 | 10 | 9.09 |
| 0.364 | 1.092 | 0.219 | 79.9 | 10 | 8.73 |
| | | Mean ± S.D | 85.0 ± 5.6 | | 8.1 ± 2.2 |

204
205 17. Centrifuge 50 ml tube labelled ‘Cells’ for 2 min, 130 *x g*, room temp.
206 18. Carefully pour off the supernatant leaving behind the loose cell pellet.
207 19. Resuspend the cell pellet with 1 ml of extracellular solution.
208 20. The cell suspension needs to be adjusted to a concentration of 1 x $10^6$/ml. This is achieved by
209 adjusting the volume to the same value as the total number of cells. (Total viable cell count = 5
210 x $10^6$, adjust cell volume to 5 ml). Centrifuge 50 ml tube labelled ‘Cells’ for 2 min, 130 *x g*, room
211 temp.
212 21. The cells are now ready to be seeded onto the Matrigel coated Seahorse XF 24 well cell plate
213 (Figure 1).
214 22. Remove the XF 24 well cell plate and remove the 100 µl serum-free Seahorse media from each
215 well.
216 23. Continuously mixing the cell suspension during seeding by pipetting up and down, transfer 75
217 µl of cell suspension into each well leaving the following cells blank to give a total of 75,000
218 murine pancreatic cells per well.
219

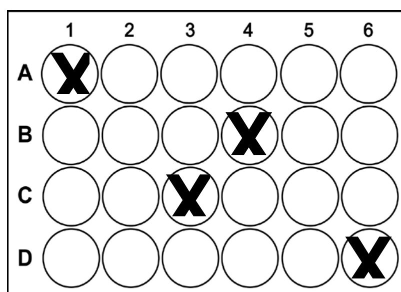


**Figure 1. Well allocation for blank well for XF24 cell plate**

24. Place the lid back onto the XF24 cell plate and place in a 37 °C $CO_2$ free incubator for 1 hour for the cells to adhere.
25. Whilst the cells are adhering the XF24 cartridge can be loaded with the compounds that comprise a mitochondrial "stress" test.
26. After one hour remove the 75 µl of extracellular solution from the cells. Quickly add 450 µl pre-warmed serum free Seahorse media to every well. Return to a 37 °C $CO_2$ free incubator until ready for use.

D. Preparing and loading compounds for the mitochondrial "stress" test

1. There are two types of assays that can be performed:
   Standard Assay – only involves the injection of modulators included in the kit.
   Modified Assay – includes an additional injection of a test compound prior to oligomycin injection, and Port A is used for the testing compound.
2. Refer to Table 2 for loading volume and port designation for compounds in different types of assays.

**Table 2. Loading regime for mitochondrial stress test**

| Port | Standard Assay | Modified Assay | Port Concentration | Port Volume |
|---|---|---|---|---|
| A | Oligomycin | Test compound | 10x | 50 |
| B | FCCP | Oligomycin | 10x | 55 |
| C | Rotenone & Antimycin A | FCCP | 10x | 62 |
| D | Empty | Rotenone & Antimycin A | 10x | 68 |

3. Make a working concentration of 10x the final required concentration required from stock solutions stored at -20 °C as follows:
   a. Oligomycin stock solution in DMSO
      10 µl in 1 ml of Seahorse media
      For a 10 x solution of 10 µg/ml for a final concentration of 1 µg/ml.
   b. Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) stock solution in DMSO

Bio-protocol 9(xx): exxxx.
DOI:10.21769/BioProtoc.xxxx

3 µl in 1 ml of Seahorse media
For a 10 x solution of 3 µM for a final concentration of 0.3 µM.
c. Rotenone stock solution in DMSO and Antimycin A stock solution in ethanol
10 µl of each in 1 ml of Seahorse media
For a 10 x solution of 20 µg/ml for a final concentration of 2 µg/ml.

4. Make a vehicle control solution for any well or port that is not receiving a compound or stress test with appropriate amounts of DMSO/Ethanol or other vehicle utilized for specific compounds.
5. Remove both the sensor plate and the corresponding utility plate together from the 37 °C $CO_2$ free incubator and remove the lid.
6. Load the corresponding volume of each compound appropriate for the assay type into the appropriate port on the Sensor plate.
7. Once all the compounds have been added to the ports, the lid can be replaced on to the Sensor plate and return to a 37 °C $CO_2$ free incubator until ready for use.

E. Oxygen consumption and lactate production analysis

1. Programme the Seahorse Extracellular Flux instrument as follows:
   a. Calibration
   b. Equilibration
   c. [Mix – 2 min, Wait – 2 min, Measure – 2 min] x 5 to ensure a stable baseline
   d. Inject Port A
   e. [Mix – 2 min, Wait – 2 min, Measure – 2 min] x 3 for standard assay oligomycin injection or x 5 typically for modified assay which gives 30 min for any test compound application.
   f. Inject Port B
   g. [Mix – 2 min, Wait – 2 min, Measure – 2 min] x 3 for standard assay FCCP injection and also x 3 for modified assay, oligomycin injection
   h. Inject Port C
   i. [Mix – 2 min, Wait – 2 min, Measure – 2 min] x 2 for standard assay Antimycin A/Rotenone injection and x 3 for modified assay, FCCP injection
   j. Inject Port D (modified assay only)
   k. [Mix – 2 min, Wait – 2 min, Measure – 2 min] x 2 for modified assay only, Antimycin A/Rotenone injection
2. After reviewing the group definitions, plate map layout, click Start Run.
3. After you enter the save location for your result file (following completion of the assay), the tray door on the XF24 Analyser will open.

   **Important! Before starting calibration, ensure:**
4. The sensor cartridge fits properly on the Utility plate.
5. The lid is removed from the sensor cartridge.
6. Proper orientation (direction) of the sensor cartridge on the Utility plate.
7. Place the sensor cartridge (hydrated and loaded with compounds) and Utility plate onto the tray

286 when prompted.
287 8. Press "Start" to initiate sensor cartridge calibration.
288 9. Time to complete calibration is approximately 10-20 min (for assays at 37°C). For XF assays
289 performed at temperatures other 37°C an additional 30 min of pre-calibration time will be added
290 to ensure accurate data acquisition.
291 10. Once sensor cartridge calibration is complete, the instrument controller will display the Load
292 Cell Plate dialogue
293 11. Click Open Tray to eject the Utility plate and load the Cell Plate on the tray. The sensor cartridge
294 remains inside the XF Analyser for this step.
295 **Important! Before loading the Cell plate, ensure:**
296 12. The lid is removed the Cell Plate.
297 13. Proper orientation (direction) of the Cell Plate on the tray.
298 14. After placing the Cell Plate on the tray, click Load Cell Plate to initiate equilibration.
299 15. After completing equilibration, the assay will automatically begin acquiring baseline
300 measurements (as outlined in your instrument protocol).
301 16. Once the final measurement command in the instrument protocol is completed, Wave Controller
302 software will display the Unload Sensor Cartridge dialog.
303 17. Click Eject when ready to eject the sensor cartridge and cell plate. Set aside for later analysis if
304 necessary (example - cell count normalization).
305 18. After removing the sensor cartridge and cell plate, the Assay Complete dialogue will appear.
306 19. Click View Results to immediately open your assay result file. Download both files for the
307 experiment
308 20. Excel file
309 21. Xfd. file
310
311 **Data analysis**
312
313 1. Open the Xfd file in Wave software.
314 2. You need to change the file format into a Prism file by selecting 'save as' and then choosing
315 the .pzfx format.
316 3. The data can now be opened directly in prism.
317 4. Each experimental condition needs at least 3 wells per plate and for statistics needs at least n
318 = 6 plates using fresh pancreas isolated from a separate mouse each time.
319 5. Exclusion criteria:
320 a. Unstable baseline, usually when the baseline shows a steady rate of decline excessively
321 low or high baseline <200 or > 800 pMol/min OCR.
322 b. Lack of response by stress test control wells to stress test compounds.
323 c. Outlier data points – entire series for a well and/or individual data points
324

Bio-protocol 9(xx): exxxx.
DOI:10.21769/BioProtoc.xxxx

325 **Notes**

326

327 **Important!** – Before you start your XF Assay

328 1. Visually inspect the injection ports for even loading. The liquid should be in the port, make sure
329 there are no residual drops on the top of the sensor cartridge.

330 2. View cells under a microscope to:

331 a. Confirm cell health, morphology, seeding uniformity and purity (no contamination).

332 b. For *adherent cells*, ensure cells are adhered with a consistent monolayer and were not
333 washed away during washing step.

334 **Recipes**

335

336 1. Extracellular solution (mM)

337 140 mM NaCl

338 4.7 mM KCl

339 1.13 mM $MgCl_2$

340 1 mM $CaCl_2$

341 10 mM D-glucose

342 10 mM HEPES (adjust to pH 7.25 using NaOH)

343 2. Seahorse media

344 a. Prepare DMEM by adding 800 ml $dH_2O$ to powdered DMEM.

345 b. Prepare assay medium by supplementing DMEM medium with 1 mM pyruvate, 2 mM
346 glutamine, and 10 mM glucose as in the table below (Table 3):

347

348 **Table 3. Recipe for serum free non-buffered Seahorse media**

| Reagent/Part Number | Final Concentration | Volume |
|---|---|---|
| DMEM Medium | - | 1.0 L |
| D-Glucose (1.0 M solution) | 10 mM | 1.8 g |
| Pyruvate (100 mM solution) | 1 mM | 10 ml |
| L-Glutamine (200 mM solution) | 2 mM | 10 ml |

349

350 c. Bring XF medium with supplements to pH 7.4 and adjust the final volume to 1 litre, transfer
351 into a Class 2 microbiological safety cabinet.

352 d. Filter with a filter pipette into sterile 50 ml aliquot centrifuge tubes. Store in a fridge for up to
353 1 month.

354

355 **Acknowledgments**

356

357 This work was supported by the Wellcome Trust (102381/Z/13/Z) and by the National Institute for
358 Health Research (UK) grant to the NIHR Liverpool Pancreas Biomedical Research Unit. This

Bio-protocol 9(xx): exxxx.
DOI:10.21769/BioProtoc.xxxx

359 protocol was first published in the original article from Armstrong *et al.* (2018).
360
361 **Competing interests**
362
363 The authors declare that they have no conflicts of interest with the contents of this article.
364
365 **Ethics**
366
367 The animals were humanely sacrificed by increasing $CO_2$ (schedule 1 procedure) in accordance
368 with the Animals (Scientific Procedures) Act (1986) under Establishment License 40/2408 with
369 approval by the University of Liverpool Animal Welfare Committee and Ethical Review Body
370 (X70548BEB and PPL 70/8109).
371
372 **References**
373

374 1. Armstrong, J. A., Cash, N. J., Morton, J. C., Tepikin, A. V., Sutton, R. and Criddle, D. N. (2019).
375 Mitochondrial targeting of antioxidants alters pancreatic acinar cell bioenergetics and
376 determines cell fate. *Int J Mol Sci* 20(7).
377 2. Armstrong, J. A., Cash, N. J., Ouyang, Y., Morton, J. C., Chvanov, M., Latawiec, D., Awais, M.,
378 Tepikin, A. V., Sutton, R. and Criddle, D. N. (2018). Oxidative stress alters mitochondrial
379 bioenergetics and modifies pancreatic cell death independently of cyclophilin D, resulting in an
380 apoptosis-to-necrosis shift. *J Biol Chem* 293(21): 8032-8047.
381 3. Criddle, D. N. (2016). Reactive oxygen species, $Ca^{2+}$ stores and acute pancreatitis; a step closer
382 to therapy? *Cell Calcium* 60(3): 180-189.
383 4. Criddle, D. N., Murphy, J., Fistetto, G., Barrow, S., Tepikin, A. V., Neoptolemos, J. P., Sutton, R.
384 and Petersen, O. H. (2006). Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol
385 trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 130(3): 781-793.
386 PM:16530519
387 5. Criddle, D. N., Raraty, M. G., Neoptolemos, J. P., Tepikin, A. V., Petersen, O. H. and Sutton, R.
388 (2004). Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid
389 metabolites. *Proc Natl Acad Sci U S A* 101(29): 10738-10743.
390 6. Habtezion, A., Gukovskaya, A. S. and Pandol, S. J. (2019). Acute pancreatitis: a multifaceted
391 set of organelle and cellularinteractions. *Gastroenterology* 156(7): 1941-1950.
392 7. Mukherjee, R., Mareninova, O. A., Odinokova, I. V., Huang, W., Murphy, J., Chvanov, M., Javed,
393 M. A., Wen, L., Booth, D. M., Cane, M. C., Awais, M., Gavillet, B., Pruss, R. M., Schaller, S.,
394 Molkentin, J. D., Tepikin, A. V., Petersen, O. H., Pandol, S. J., Gukovsky, I., Criddle, D. N.,
395 Gukovskaya, A. S., Sutton, R. and Unit, N. P. B. R. (2016). Mechanism of mitochondrial
396 permeability transition pore induction and damage in the pancreas: inhibition prevents acute
397 pancreatitis by protecting production of ATP. *Gut* 65(8): 1333-1346.

Bio-protocol 9(xx): exxxx.
DOI:10.21769/BioProtoc.xxxx

398 8. Voronina, S., Sukhomlin, T., Johnson, P. R., Erdemli, G., Petersen, O. H. and Tepikin, A. (2002).
399 Correlation of NADH and $Ca^{2+}$ signals in mouse pancreatic acinar cells. *J.Physiol* 539(Pt 1): 41-
400 52.
401 9. Voronina, S. G., Barrow, S. L., Simpson, A. W., Gerasimenko, O. V., da Silva Xavier, G., Rutter,
402 G. A., Petersen, O. H. and Tepikin, A. V. (2010). Dynamic changes in cytosolic and mitochondrial
403 ATP levels in pancreatic acinar cells. *Gastroenterology* 138(5): 1976-1987.
404