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KAPP-Sen TMC: Single Cell Dissociation of Human Placenta, Fetal Membranes and Umbilical Cord

 Forked from [JAX-Sen: Mouse placenta single-cell dissociation for single-cell RNA sequencing](#)

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We use this protocol and it's working

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Abstract

Placenta, fetal membranes and umbilical cords are obtained from C-section deliveries across different age groups and collected in MACS buffer and processed the next day. This protocol is used to generate single cells from tissue samples from the placenta (basal and chorionic plates), fetal membranes (amnio and chorionic membranes) and the umbilical cord. It involves steps such as enzymatic digestions, ACK lysis and careful filtration to preserve the single cells obtained. Before submission for scRNAseq, the samples are sorted and enriched for live cells to eliminate cellular debris. For the placental samples (basal plate and chorionic plate), the samples are also enriched for non-immune cells using CD45+ depletion.

Single Cell Dissociation of Placenta (Basal Plate and Chorionic Plate):

- 1 Place BP and CP on ice after getting it from the package.
- 2 Decant the MACS buffer from the tube carefully and add fresh cold PBS and wash the tissue by gently moving it inside the tube.
Do this step TWICE to get rid of the blood as much as possible.
- 3 Place tissues on 10cm petri dishes and take a picture for size and texture reference.
- 4 Start mincing the tissues *gently* on ice, to make pieces of size $<1\text{mm}^3$.
- 5 Transfer the minced tissues into C-tubes by tilting the plates and pouring the soup. Wash the plate with 0.5ml digest media and add it to the tube.
- 6 Depending on the volume of the minced tissue, add digest media.
Eg. 5 ml minced tissue needs 5 ml digest media.
Digest Media: 15 ml
Dispase: 3.6 ml
Collagenase 1: 150 uL
DNase: 22.5 uL
DMEM: 11.4 ml
- 7 Mix the tube gently TWICE by inverting and place them in the rotor shaker (250 rpm) at 37 degrees.
- 8 Incubation time: 1 hour, with *checking every 15 mins* to check for digestion based on minced tissue size.
- 9 Just close to 1 hour end, prepare the 70 um MACS smart strainers and 50 ml tubes ready for filtration using PBS+BSA.
- 10 Take the tubes out of the rotor and filter the digest through the filter gently. If there is clogging, gently scrape the clog to enable filtration.
- 11 Fill the 50 ml tube with Stop buffer (10% FBS in DMEM) to stop the digestion.



- 12 Transfer the retentate back to the C-tube, added equal volume of digest media and leave for 10 more mins (*or more based on sample observation*) and do Gentle MACS octo-dissociation (h_cord_01) TWICE.
This method causes foam/bubbles.
- 13 During this time, prepare a fresh filter+50 ml tube for each sample with PBS+BSA coating.
- 14 Filter the new digest through the filter gently, avoiding foam/bubble that might dry out and kill cells. If there is clogging, gently scrape the clog to enable filtration.
- 15 Fill the 50 ml tube with Stop buffer to stop the digestion.
- 16 Now there will be 2 tubes for BP and 2 tubes for CP, both with 50 ml stop buffer+cells.
- 17 Centrifuge the 4 tubes: 800g, 5 min, 4 degrees.
- 18 Decant supernatant carefully, resuspend and pool the samples using cold PBS into 2 tubes: one for BP and one for CP.
- 19 Centrifuge the 4 tubes: 800g, 5 min, 4 degrees.
- 20 ACK lysis step: Add ACK lysis buffer, 5-7 times volume of the cell pellet and incubate at RT for 10 mins.
- 21 Add PBS+MACS buffer to stop the reaction, centrifuge the 4 tubes: 800g, 5 min, 4 degrees.
- 22 If pellet is still red, repeat ACK lysis with 5-7 times volume in RT for 10 mins.
- 23 Fill the tubes with PBS+MACS and wash the cells by pipetting with wide-bore pipette gently.
- 24 Centrifuge the 2 tubes: 800g, 5 min, 4 degrees.
During this time, prepare 2 ml tubes by labeling and coating with PBS+MACS.



- 25 If pellet is white/cream, resuspend the pellet in 1 ml PBS+MACS and transfer to the 2 ml tubes.
- 26 Count cells by AOPI staining.
- 27 Proceed with Cell staining:
 - 27.1 Pellet the cells by centrifuge: 800g, 5 min, 4 degrees.
 - 27.2 Add 45uL FC block to the pellet. If pellet is large (larger than 45 uL), *double or triple the volume as needed while going forward.*
 - 27.3 Incubate with FC block on ice for 5 mins.
 - 27.4 Add CD45-488 antibody (5uL per 45uL FC block)-so double or triple the volume accordingly. Gently pipette cells up and down with coated tip.
 - 27.5 Incubate on ice for 25 mins.
 - 27.6 Fill the tube with PBS+BSA and wash the cells gently.
 - 27.7 Centrifuge the 2 tubes: 800g, 5 min, 4 degrees. During this time, prep and coat Flow Sorting Tubes.
 - 27.8 Decant supernatant carefully using a pipette and resuspend pellets in 300 uL Cell Staining buffer.
 - 27.9 Add 10 uL Calcein and 10uL PI and stain for 15-20 mins.
- 28 Sort for live CD45- cells/CD45+ cells and submit for scRNAseq.

Single Cell Dissociation of Membranes and Umbilical Cord:

- 29 Place AM, CM and UC on ice after getting it from the package.
- 30 Decant the MACS buffer from the tube carefully and add fresh cold PBS and wash the tissue by gently moving it inside the tube.
Do this step TWICE to get rid of the blood as much as possible.
- 31 Place tissues on 10cm petri dishes, dissect out any 'villous regions' attached to the membrane and take a picture for size and texture reference.
- 32 Start mincing the tissues *gently* on ice, and transfer to C-tubes as necessary by tilting the plates and pouring the soup, to make pieces of size $<1\text{mm}^3$.
- 33 Wash the plate with 0.5ml Buffer L and add it to the tube and add 3.4 ml Buffer L.
- 34 Add enzymes from the Umbilical Cord Dissociation Kit, human (Miltenyi) (per sample):
Enzyme B: 8 uL
Enzyme D: 200 uL
Enzyme A: 20 uL
Enzyme P: 125 uL
- 35 Mix the tube gently TWICE by inverting and place them in the rotor shaker (250 rpm) at 37 degrees.
- 36 Incubation time: 1 hour , with *checking every 15 mins* to check for digestion based on minced tissue size of AM and CM, shorten the time if needed. Conversely, extend the time for UC to 1:30 hours if needed.
- 37 Just close to incubation end for the 3 samples respectively, prepare the 70 um MACS smart strainers and 50ml tubes ready for using PBS+BSA.
- 38 Take the AM and CM out of the rotor and filter the digest through the filter gently. If there is clogging, gently scrape the clog to enable filtration.
- 39 Fill the 50 ml tube with Stop buffer to stop the digestion. Leave the 2 samples on ice.
- 40 For UC, after incubation, do Gentle MACS octo-dissociation (h_cord_01) ONCE. This method might cause foam/bubbles.

- 41 During this time, prepare a fresh filter+50 ml tube for each sample with PBS+BSA coating.
- 42 Filter the UC digest through the filter gently, avoiding foam/bubble that might dry out and kill cells. If there is clogging, gently scrape the clog to enable filtration.
- 43 Fill the 50 ml tube with Stop buffer to stop the digestion.
- 44 If there is retentate, transfer the retentate back to the C-tube, added equal volume of digest media and leave for 10 more minutes (*or more based on sample observation*) and do Gentle MACS octo-dissociation (h_cord_01) TWICE. This method causes foam/bubbles.
- 45 During this time, prep a fresh filter+50 ml tube for each sample with PBS+BSA coating.
- 46 Filter the UC digest through the filter gently, avoiding foam/bubble that might dry out and kill cells. If there is clogging, gently scrape the clog to enable filtration.
- 47 Fill the 50 ml tube with Stop buffer to stop the digestion.
- 48 Centrifuge the 4 tubes: 800g, 5 min, 4 degrees.
- 49 Decant supernatant carefully, resuspend and pool the samples using cold PBS into 3 tubes: one for AM, one for CM and one for UC.
- 50 Centrifuge the 3 tubes: 800g, 5 min, 4 degrees.
- 51 ACK lysis step: Add ACK lysis buffer, 5-7 times volume of the cell pellet and incubate at RT for 10 mins.
- 52 Add PBS+MACS buffer to stop the reaction, centrifuge the 3 tubes: 800g, 5 min, 4 degrees.
- 53 If pellet is still red, repeat ACK lysis with 5-7 times volume in RT for 10 mins.



- 54 Fill the tubes with PBS+MACS and wash the cells by pipetting with wide-bore pipette gently.
- 55 Centrifuge the tubes: 800g, 5 min, 4 degrees.
During this time, prepare 2 ml tubes by labeling and coating with PBS+MACS.
- 56 If the pellet is white/cream, resuspend the pellet in 1 ml PBS+MACS and transfer to the 2 ml tubes.
- 57 Count cells by AOPI staining.
- 58 Proceed with Cell staining.
 - 58.1 Pellet the cells by centrifuge: 800g, 5 min, 4 degrees.
 - 58.2 Add 45uL FC block to the pellet. If pellet is large (larger than 45 uL), *double or triple the volume as needed while going forward.*
 - 58.3 Incubate with FC block on ice for 5 mins.
 - 58.4 Add CD45-488 antibody (5uL per 45uL FC block)-so double or triple the volume accordingly. Gently pipette cells up and down with coated tip.
 - 58.5 Incubate on ice for 25 mins.
 - 58.6 Fill the tube with PBS+BSA and wash the cells gently.
 - 58.7 Centrifuge the 2 tubes: 800g, 5 min, 4 degrees. During this time, prep and coat Flow Sorting Tubes.
 - 58.8 Decant supernatant carefully using a pipette and resuspend pellets in 300 uL Cell Staining buffer. Add 10 uL Calcein and 10uL PI and stain for 15-20 mins.
- 59 Sort for live cells and submit for scRNAseq.

