WEBVTT

NOTE duration:"00:15:43.5090000"

NOTE language:en-us

NOTE Confidence: 0.8298683

00:00:02.950 --> 00:00:05.008 Title of this training is enriching.

NOTE Confidence: 0.8298683

00:00:05.010 --> 00:00:06.390 Rare populations of primary

NOTE Confidence: 0.8298683

00:00:06.390 --> 00:00:08.109 human CD 34 positive cells

NOTE Confidence: 0.8298683

00:00:08.110 --> 00:00:10.462 by fax and has been prepared by members

NOTE Confidence: 0.8298683

00:00:10.462 --> 00:00:13.391 of the Kraus lab at Yale School of

NOTE Confidence: 0.8298683

00:00:13.391 --> 00:00:15.315 Medicine in partial fulfillment of

NOTE Confidence: 0.8298683

00:00:15.315 --> 00:00:17.045 the educational aims outlined in

NOTE Confidence: 0.8298683

00:00:17.045 --> 00:00:19.298 the mission of the Yale Cooperative

NOTE Confidence: 0.8298683

00:00:19.298 --> 00:00:21.468 Center for Excellence in Hematology.

NOTE Confidence: 0.8298683

00:00:21.470 --> 00:00:24.590 The purpose of this protocol is to analyze

NOTE Confidence: 0.8298683

00:00:24.590 --> 00:00:27.440 an isolate where populations of CD 34,

NOTE Confidence: 0.8298683

00:00:27.440 --> 00:00:29.032 positive matter, poetic stem,

NOTE Confidence: 0.8298683

00:00:29.032 --> 00:00:31.420 and progenitor cells from human G,

NOTE Confidence: 0.8298683

00:00:31.420 --> 00:00:33.192 CSF, mobilized peripheral blood,

NOTE Confidence: 0.8298683

00:00:33.192 --> 00:00:35.407 but maybe more broadly applicable

NOTE Confidence: 0.8298683

00:00:35.407 --> 00:00:37.967 to other starting cell populations.

NOTE Confidence: 0.8298683

00:00:37.970 --> 00:00:40.020 We will demonstrate the sorting

NOTE Confidence: 0.8298683

00:00:40.020 --> 00:00:41.660 strategy for human megakaryocytic

NOTE Confidence: 0.8298683

00:00:41.660 --> 00:00:43.597 over three progenitors in this video

NOTE Confidence: 0.8298683

00:00:43.597 --> 00:00:45.867 as an example of a rare population

NOTE Confidence: 0.8298683

00:00:45.867 --> 00:00:48.105 within the CD 34 positive fraction.

NOTE Confidence: 0.88397837

00:00:50.300 --> 00:00:52.562 Human CD 34 positive cells are

NOTE Confidence: 0.88397837

00:00:52.562 --> 00:00:54.427 a heterogeneous mixture of stem

NOTE Confidence: 0.88397837

00:00:54.427 --> 00:00:56.882 and progenitor cells at various

NOTE Confidence: 0.88397837

00:00:56.882 --> 00:00:58.394 stages of differentiation.

NOTE Confidence: 0.88397837

00:00:58.400 --> 00:01:00.674 To study a specific population of

NOTE Confidence: 0.88397837

00:01:00.674 --> 00:01:02.970 cells like high metabolic stem cells,

NOTE Confidence: 0.88397837

00:01:02.970 --> 00:01:06.066 this population must be enriched prior

NOTE Confidence: 0.88397837

00:01:06.066 --> 00:01:09.578 to any assets that may take place.

NOTE Confidence: 0.88397837

00:01:09.580 --> 00:01:11.540 Each progenitor population is

NOTE Confidence: 0.88397837

00:01:11.540 --> 00:01:13.990 defined by a combination of

NOTE Confidence: 0.88397837

00:01:13.990 --> 00:01:16.599 surface marker expression profiles.

NOTE Confidence: 0.88397837

00:01:16.600 --> 00:01:18.692 Fluorescence activated cell sorting

NOTE Confidence: 0.88397837

00:01:18.692 --> 00:01:21.830 or fax uses fluorescent markers to

NOTE Confidence: 0.88397837

00:01:21.905 --> 00:01:24.995 detect and separate the cells with

NOTE Confidence: 0.88397837

00:01:24.995 --> 00:01:27.055 desired surface marker expression.

NOTE Confidence: 0.88397837

00:01:27.060 --> 00:01:31.386 Building a customized flow cytometry panel.

NOTE Confidence: 0.88397837

00:01:31.390 --> 00:01:34.631 Designing a multi color panel for flow

NOTE Confidence: 0.88397837

00:01:34.631 --> 00:01:36.980 cytometry takes advanced preparation.

NOTE Confidence: 0.88397837

00:01:36.980 --> 00:01:40.697 Factors that need to be considered include.

NOTE Confidence: 0.88397837

00:01:40.700 --> 00:01:42.500 Configuration of the instrument.

NOTE Confidence: 0.88397837

00:01:42.500 --> 00:01:45.200 Such as the lasers and filters.

NOTE Confidence: 0.88397837

00:01:45.200 --> 00:01:49.766 It is equipped with. The number of

NOTE Confidence: 0.88397837

00:01:49.766 --> 00:01:53.438 colors or antibodies to be used.

NOTE Confidence: 0.88397837

00:01:53.440 --> 00:01:57.070 And the abundance of the antigens.

NOTE Confidence: 0.88397837

00:01:57.070 --> 00:02:00.514 We highly recommend utilizing Flora Finder as

NOTE Confidence: 0.88397837

00:02:00.514 --> 00:02:04.537 a platform for designing a multi color panel.

NOTE Confidence: 0.88397837

00:02:04.540 --> 00:02:07.316 Floor Finder allows the user to enter the

NOTE Confidence: 0.88397837

00:02:07.316 --> 00:02:09.139 exact specifications of their cytometer,

NOTE Confidence: 0.88397837

00:02:09.140 --> 00:02:11.235 including which lasers and filter

NOTE Confidence: 0.88397837

00:02:11.235 --> 00:02:13.980 sets are installed in the machine.

NOTE Confidence: 0.88397837

00:02:13.980 --> 00:02:14.400 Next,

NOTE Confidence: 0.88397837

00:02:14.400 --> 00:02:16.920 users can search and select which

NOTE Confidence: 0.88397837

00:02:16.920 --> 00:02:19.096 cell surface antigens and or

NOTE Confidence: 0.88397837

00:02:19.096 --> 00:02:20.776 fluorescent proteins they intend

NOTE Confidence: 0.88397837

00:02:20.776 --> 00:02:23.289 to analyze their cell sample for.

NOTE Confidence: 0.88397837

00:02:23.290 --> 00:02:25.546 It is useful to have prior knowledge of

NOTE Confidence: 0.88397837

00:02:25.546 --> 00:02:27.578 the antigen abundance on the cells in

NOTE Confidence: 0.88397837

00:02:27.578 --> 00:02:29.710 your sample and cell type of interest,

NOTE Confidence: 0.88397837

00:02:29.710 --> 00:02:32.470 but it is not necessary.

NOTE Confidence: 0.88397837

00:02:32.470 --> 00:02:34.455 Based on the specifications of

NOTE Confidence: 0.88397837

00:02:34.455 --> 00:02:36.884 the CYTOMETER and antigens to be

NOTE Confidence: 0.88397837

00:02:36.884 --> 00:02:38.759 detected that the user entered,

NOTE Confidence: 0.88397837

00:02:38.760 --> 00:02:41.172 Flora Finder populates a chart of

NOTE Confidence: 0.88397837

00:02:41.172 --> 00:02:42.378 commercially available antibodies

NOTE Confidence: 0.88397837

00:02:42.378 --> 00:02:44.493 for those antigens categorized by

NOTE Confidence: 0.88397837

00:02:44.493 --> 00:02:46.558 fluorescent channel such that the

NOTE Confidence: 0.88397837

00:02:46.558 --> 00:02:49.142 selection of 1 antibody in a particular

NOTE Confidence: 0.88397837

00:02:49.142 --> 00:02:50.546 channel automatically blocks the

NOTE Confidence: 0.88397837

00:02:50.550 --> 00:02:52.122 user from selecting additional

NOTE Confidence: 0.88397837

00:02:52.122 --> 00:02:54.087 antibodies in the same channel,

NOTE Confidence: 0.88397837

00:02:54.090 --> 00:02:57.122 thus reducing spectral overlap.

NOTE Confidence: 0.88397837

00:02:57.122 --> 00:02:59.396 Titrating staining antibodies.

NOTE Confidence: 0.88397837

00:02:59.400 --> 00:03:01.435 To ensure appropriate staining but

NOTE Confidence: 0.88397837

00:03:01.435 --> 00:03:03.063 not compromise antibody specificity,

NOTE Confidence: 0.88397837

00:03:03.070 --> 00:03:06.136 it is recommended that you titrate

NOTE Confidence: 0.88397837

00:03:06.136 --> 00:03:08.686 your antibodies prior to using

NOTE Confidence: 0.88397837

00:03:08.686 --> 00:03:11.570 them in a flow analysis or sort.

NOTE Confidence: 0.88397837

00:03:11.570 --> 00:03:13.500 To accomplish this utiliza cell

NOTE Confidence: 0.88397837

00:03:13.500 --> 00:03:15.842 sample that contains a mixture of

NOTE Confidence: 0.88397837

00:03:15.842 --> 00:03:17.900 cells that are positive for the

NOTE Confidence: 0.88397837

00:03:17.900 --> 00:03:19.490 antigen and negative pipette.

NOTE Confidence: 0.88397837

00:03:19.490 --> 00:03:22.618 A constant number of cells in a constant

NOTE Confidence: 0.88397837

00:03:22.618 --> 00:03:25.302 volume of staining solution into at

NOTE Confidence: 0.88397837

00:03:25.302 --> 00:03:28.580 least three but preferably 5 fax chips.

NOTE Confidence: 0.88397837

00:03:28.580 --> 00:03:31.106 Keep one tube as the unstained

NOTE Confidence: 0.88397837

00:03:31.106 --> 00:03:33.280 control and add increasing volumes

NOTE Confidence: 0.88397837

00:03:33.280 --> 00:03:36.171 of the antibody wish to titer to

NOTE Confidence: 0.88397837

00:03:36.171 --> 00:03:37.450 each subsequent tube.

NOTE Confidence: 0.88397837

00:03:37.450 --> 00:03:39.370 The range of volumes you choose

NOTE Confidence: 0.88397837

00:03:39.370 --> 00:03:41.727 should be based on the recommended

NOTE Confidence: 0.88397837

00:03:41.727 --> 00:03:43.735 dilution from the manufacturer.

NOTE Confidence: 0.88397837

00:03:43.740 --> 00:03:44.764 For example.

NOTE Confidence: 0.88397837

00:03:44.764 --> 00:03:47.836 If the recommended volume test for

NOTE Confidence: 0.88397837

00:03:47.836 --> 00:03:50.299 the manufacturer is 5 microliters,

NOTE Confidence: 0.88397837

00:03:50.300 --> 00:03:52.990 pipette 5 microliters of antibody

NOTE Confidence: 0.88397837

00:03:52.990 --> 00:03:54.604 into one tube.

NOTE Confidence: 0.88397837

00:03:54.610 --> 00:03:57.050 Two microliters of antibody into

NOTE Confidence: 0.88397837

00:03:57.050 --> 00:03:58.514 the next tube.

NOTE Confidence: 0.88397837

00:03:58.520 --> 00:04:00.800 One microliter of antibody into

NOTE Confidence: 0.88397837

00:04:00.800 --> 00:04:02.168 the next tube.

NOTE Confidence: 0.88397837

00:04:02.170 --> 00:04:05.008 And half a microliter of antibody

NOTE Confidence: 0.88397837

00:04:05.008 --> 00:04:06.900 into the last tube.

NOTE Confidence: 0.88397837

00:04:06.900 --> 00:04:08.716 At the Cytometer record,

NOTE Confidence: 0.88397837

00:04:08.716 --> 00:04:11.930 at least 10,000 events for each tube.

NOTE Confidence: 0.88397837

00:04:11.930 --> 00:04:13.935 Calculate the staining index by

NOTE Confidence: 0.88397837

00:04:13.935 --> 00:04:16.819 measuring the MFA of the negative cells

NOTE Confidence: 0.88397837

00:04:16.819 --> 00:04:19.225 and the standard deviation as well

NOTE Confidence: 0.88397837

00:04:19.225 --> 00:04:22.039 as the MFA for the positive cells.

NOTE Confidence: 0.88397837

00:04:22.040 --> 00:04:23.912 With these three measurements,

NOTE Confidence: 0.88397837

00:04:23.912 --> 00:04:25.784 calculate the staining index

NOTE Confidence: 0.88397837

00:04:25.784 --> 00:04:28.054 by subtracting the MFA of the

NOTE Confidence: 0.88397837

00:04:28.054 --> 00:04:29.634 negative population from the MFA

NOTE Confidence: 0.88397837

00:04:29.634 --> 00:04:31.538 of the positive population.

NOTE Confidence: 0.88397837

00:04:31.540 --> 00:04:33.634 And divide this by two times

NOTE Confidence: 0.88397837

00:04:33.634 --> 00:04:35.030 the standard deviation of

NOTE Confidence: 0.8527813

00:04:35.103 --> 00:04:37.365 the MFA of the negative population.

NOTE Confidence: 0.8527813

00:04:37.370 --> 00:04:39.476 Plot the staining index for each

NOTE Confidence: 0.8527813

00:04:39.476 --> 00:04:42.879 tube on a graph to identify the best

NOTE Confidence: 0.8527813

00:04:42.879 --> 00:04:45.279 dilution for that particular antibody.

NOTE Confidence: 0.8527813

00:04:45.280 --> 00:04:48.577 Sawing and staining CD. 34 positive cells.

NOTE Confidence: 0.84104687

00:04:50.890 --> 00:04:53.767 For the purposes of this didactic video,

NOTE Confidence: 0.84104687

00:04:53.770 --> 00:04:56.885 we will demonstrate the staining protocol for

NOTE Confidence: 0.84104687

00:04:56.885 --> 00:04:58.990 human megakaryocytic erythroid progenitors.

NOTE Confidence: 0.84104687

00:04:58.990 --> 00:05:00.490 You will need to substitute

NOTE Confidence: 0.84104687

00:05:00.490 --> 00:05:01.690 your antibodies of interest,

NOTE Confidence: 0.84104687

00:05:01.690 --> 00:05:04.810 but the protocol remains the same.

NOTE Confidence: 0.84104687

00:05:04.810 --> 00:05:06.078 With stained with CD,

NOTE Confidence: 0.84104687

00:05:06.078 --> 00:05:08.455 34 positive cells with a panel of

NOTE Confidence: 0.84104687

00:05:08.455 --> 00:05:11.054 seven antibodies, one of which is

NOTE Confidence: 0.84104687

00:05:11.054 --> 00:05:13.730 a cocktail of Lenny edge markers.

NOTE Confidence: 0.84104687

00:05:13.730 --> 00:05:16.386 To reduce the number of channels required for

NOTE Confidence: 0.84104687

00:05:16.386 --> 00:05:19.148 the sort and thus reduce spectral overlap,

NOTE Confidence: 0.84104687

00:05:19.150 --> 00:05:21.310 we use antibodies against the lineages.

NOTE Confidence: 0.84104687

00:05:21.310 --> 00:05:23.138 Markers that are directly

NOTE Confidence: 0.84104687

00:05:23.138 --> 00:05:24.509 conjugated to biotin.

NOTE Confidence: 0.84104687

00:05:24.510 --> 00:05:26.405 So we incorporate a secondary

NOTE Confidence: 0.84104687

00:05:26.405 --> 00:05:27.921 stain with streptavidin antibody

NOTE Confidence: 0.84104687

00:05:27.921 --> 00:05:29.539 directly conjugated to a floor.

NOTE Confidence: 0.84104687

00:05:29.540 --> 00:05:31.580 For that has limited spectral overlap

NOTE Confidence: 0.84104687

00:05:31.580 --> 00:05:34.198 with the rest of our staining panel.

NOTE Confidence: 0.63781935

00:05:37.090 --> 00:05:41.030 Required reagents include FBZ.

NOTE Confidence: 0.63781935

00:05:41.030 --> 00:05:44.578 Buffer one. Fax buffer.

NOTE Confidence: 0.63781935

00:05:44.578 --> 00:05:46.750 And titrated antibodies.

NOTE Confidence: 0.813827

00:05:50.840 --> 00:05:52.920 Prepare labeled facts tubes

NOTE Confidence: 0.813827

00:05:52.920 --> 00:05:55.000 for each compensation control.

NOTE Confidence: 0.813827

00:05:55.000 --> 00:05:56.880 Fluorescence minus one control.

NOTE Confidence: 0.813827

00:05:56.880 --> 00:05:59.700 As well as the sample tube.

NOTE Confidence: 0.8699426

00:06:03.120 --> 00:06:05.288 Compensation controls are cells

NOTE Confidence: 0.8699426

00:06:05.288 --> 00:06:07.998 stained with a single antibody.

NOTE Confidence: 0.8699426

00:06:08.000 --> 00:06:10.185 Compensation controls are required for

NOTE Confidence: 0.8699426

00:06:10.185 --> 00:06:13.413 every floor floor and allow the cytometry

NOTE Confidence: 0.8699426

00:06:13.413 --> 00:06:15.833 software to calculate and subtract

NOTE Confidence: 0.8699426

00:06:15.833 --> 00:06:18.150 spectral overlap between flora force.

NOTE Confidence: 0.8699426

00:06:18.150 --> 00:06:19.690 Fluorescence minus one controls

NOTE Confidence: 0.8699426

00:06:19.690 --> 00:06:22.375 are cells stained with all but one

NOTE Confidence: 0.8699426

00:06:22.375 --> 00:06:24.235 of the antibodies in the panel.

NOTE Confidence: 0.8699426

00:06:24.240 --> 00:06:27.536 It identifies the true negative of a floor

NOTE Confidence: 0.8699426

00:06:27.536 --> 00:06:29.786 for considering all the interference

NOTE Confidence: 0.8699426

00:06:29.786 --> 00:06:32.486 from the other staining floor force.

NOTE Confidence: 0.8699426

00:06:32.490 --> 00:06:34.650 These controls help determine the

NOTE Confidence: 0.8699426

00:06:34.650 --> 00:06:37.656 location of gates that separates cells not

NOTE Confidence: 0.8699426

00:06:37.656 --> 00:06:40.358 expressing the antigen from cells that are.

NOTE Confidence: 0.8699426

00:06:40.360 --> 00:06:42.880 They are required if the expression

NOTE Confidence: 0.8699426

00:06:42.880 --> 00:06:45.467 pattern of the antigen in your

NOTE Confidence: 0.8699426

00:06:45.467 --> 00:06:47.532 starting population is a continuum

NOTE Confidence: 0.8699426

00:06:47.532 --> 00:06:50.009 of negative to high expressing.

NOTE Confidence: 0.8699426

00:06:50.010 --> 00:06:52.827 Or the expression of the antigen is very low,

NOTE Confidence: 0.8699426

00:06:52.830 --> 00:06:54.372 so the positive signal is just

NOTE Confidence: 0.8699426

00:06:54.372 --> 00:06:55.850 slightly higher than the background

NOTE Confidence: 0.8699426

00:06:55.850 --> 00:06:57.840 fluorescence of the negative population.

NOTE Confidence: 0.8290179

00:06:59.710 --> 00:07:01.760 We quickly thaw cryopreserved CD

NOTE Confidence: 0.8290179

00:07:01.760 --> 00:07:04.629 34 positive cells in a 37 degree

NOTE Confidence: 0.8290179

00:07:04.629 --> 00:07:07.233 water bath until just a few ice

NOTE Confidence: 0.8290179

00:07:07.233 --> 00:07:09.546 crystals are left in the cryotube.

NOTE Confidence: 0.8290179

00:07:09.550 --> 00:07:11.566 Take care not to submerge the

NOTE Confidence: 0.8290179

00:07:11.566 --> 00:07:14.214 O-ring and cap of the cryo vial

NOTE Confidence: 0.8290179

00:07:14.214 --> 00:07:16.269 to reduce risk of contamination.

NOTE Confidence: 0.8290179

00:07:16.270 --> 00:07:18.058 When the cells are nearly thought,

NOTE Confidence: 0.8290179

00:07:18.060 --> 00:07:20.160 spray the cry of I'll with 70%

NOTE Confidence: 0.8290179

00:07:20.160 --> 00:07:22.434 ethanol and wipe it dry before

NOTE Confidence: 0.8290179

00:07:22.434 --> 00:07:24.998 bringing it into the hood to open.

NOTE Confidence: 0.8290179

00:07:25.000 --> 00:07:26.810 Be sure to practice strict

NOTE Confidence: 0.8290179

00:07:26.810 --> 00:07:29.036 aseptic technique in a bio safety

NOTE Confidence: 0.8290179

00:07:29.036 --> 00:07:31.430 cabinet rated for BSL 2 for the

NOTE Confidence: 0.8290179

00:07:31.430 --> 00:07:33.140 duration of this protocol.

NOTE Confidence: 0.7869187

00:07:40.600 --> 00:07:42.530 Add one milliliter of 100%

NOTE Confidence: 0.7869187

00:07:42.530 --> 00:07:45.218 FBS to a 50 mil conical tube.

NOTE Confidence: 0.7208567

00:07:50.700 --> 00:07:53.557 Add one milliliter of 100% FBS to the

NOTE Confidence: 0.7208567

00:07:53.557 --> 00:07:55.711 cryotube and gently transfer the full

NOTE Confidence: 0.7208567

00:07:55.711 --> 00:07:57.877 contents of the cryotube into the

NOTE Confidence: 0.7208567

00:07:57.877 --> 00:07:59.930 50 mil conical tube containing FBS.

NOTE Confidence: 0.7640226

00:08:13.040 --> 00:08:15.525 Add up to 50 milliliters of buffer.

NOTE Confidence: 0.7640226

00:08:15.530 --> 00:08:16.954 One dropwise cell suspension

NOTE Confidence: 0.7640226

00:08:16.954 --> 00:08:19.090 in the 50 mil conical tube.

NOTE Confidence: 0.7640226

00:08:19.090 --> 00:08:22.294 To delete out the DMSO in the freezing media.

NOTE Confidence: 0.8578139

00:08:25.410 --> 00:08:28.170 Spin at 1200 RPM 4 degrees

NOTE Confidence: 0.8578139

00:08:28.170 --> 00:08:30.010 Celsius for 10 minutes.

NOTE Confidence: 0.8364337

00:08:32.860 --> 00:08:34.540 When the spin is done,

NOTE Confidence: 0.8364337

00:08:34.540 --> 00:08:35.880 carefully discard the supernatant.

NOTE Confidence: 0.7796592

00:08:40.300 --> 00:08:42.610 Resuspend the pellet with 20

NOTE Confidence: 0.7796592

00:08:42.610 --> 00:08:44.458 milliliters of fax buffer.

NOTE Confidence: 0.7796592

00:08:44.460 --> 00:08:46.315 Take a representative 10 microliter

NOTE Confidence: 0.7796592

00:08:46.315 --> 00:08:48.783 aliquot for cell counting and repeat the

NOTE Confidence: 0.7796592

00:08:48.783 --> 00:08:50.975 spin of the conical tube at 1200 RPM,

NOTE Confidence: 0.7796592

00:08:50.980 --> 00:08:52.936 4 degrees Celsius for 10 minutes.

NOTE Confidence: 0.78864664

00:08:54.970 --> 00:08:57.930 Add 10 microliters of two extra pen blue

NOTE Confidence: 0.78864664

00:08:57.930 --> 00:09:01.588 to the aliquot of cells and pipette to mix.

NOTE Confidence: 0.78864664

00:09:01.590 --> 00:09:03.360 Calculate the viable cell number

NOTE Confidence: 0.78864664

00:09:03.360 --> 00:09:05.130 in the sample control tubes.

NOTE Confidence: 0.78864664

00:09:05.130 --> 00:09:08.883 Each require 5 * 10 to the four cells.

NOTE Confidence: 0.78864664

00:09:08.890 --> 00:09:10.732 FMO tubes require as few as

NOTE Confidence: 0.78864664

00:09:10.732 --> 00:09:14.880 2 * 10 to the 4th cells.

NOTE Confidence: 0.78864664

00:09:14.880 --> 00:09:16.956 When the second spin is complete,

NOTE Confidence: 0.78864664

00:09:16.960 --> 00:09:18.565 resuspend the pellet in 500

NOTE Confidence: 0.78864664

00:09:18.565 --> 00:09:20.170 microliters of Facs buffer an

NOTE Confidence: 0.78864664

00:09:20.233 --> 00:09:22.063 aliquot the necessary volume of

NOTE Confidence: 0.78864664

00:09:22.063 --> 00:09:23.893 cells required for control tubes.

NOTE Confidence: 0.78864664

00:09:23.900 --> 00:09:25.665 Add additional facts buffer to

NOTE Confidence: 0.78864664

00:09:25.665 --> 00:09:27.816 bring the sample tube volume back

NOTE Confidence: 0.78864664

00:09:27.816 --> 00:09:29.108 up to 500 microliters.

NOTE Confidence: 0.78864664

00:09:29.110 --> 00:09:31.672 Take care not to exceed a cell

NOTE Confidence: 0.78864664

00:09:31.672 --> 00:09:34.276 concentration of 20 * 10 to the six

NOTE Confidence: 0.78864664

00:09:34.276 --> 00:09:36.740 cells per milliliter in the sample tube.

NOTE Confidence: 0.78377306

00:09:39.390 --> 00:09:41.058 Aliquot the titrated amount

NOTE Confidence: 0.78377306

00:09:41.058 --> 00:09:42.726 of primary staining antibodies

NOTE Confidence: 0.78377306

00:09:42.726 --> 00:09:44.869 into the corresponding tubes.

NOTE Confidence: 0.78377306

00:09:44.870 --> 00:09:46.920 Be especially careful when allocating

NOTE Confidence: 0.78377306

00:09:46.920 --> 00:09:49.405 antibodies in the FMO tube so

NOTE Confidence: 0.78377306

00:09:49.405 --> 00:09:51.230 you don't accidentally add the

NOTE Confidence: 0.78377306

00:09:51.230 --> 00:09:53.210 antibody that should be absent.

NOTE Confidence: 0.78377306

00:09:53.210 --> 00:09:54.690 Additionally, it is important to

NOTE Confidence: 0.78377306

00:09:54.690 --> 00:09:56.617 note that the volume of antibodies

NOTE Confidence: 0.78377306

00:09:56.617 --> 00:09:58.567 added cannot exceed the maximum

NOTE Confidence: 0.78377306

00:09:58.567 --> 00:10:00.326 standing volume, which is.

NOTE Confidence: 0.78377306

00:10:00.326 --> 00:10:02.278 100 microliters for controls

NOTE Confidence: 0.78377306

00:10:02.278 --> 00:10:04.880 and one milliliter for sample.

NOTE Confidence: 0.78377306

00:10:04.880 --> 00:10:06.008 In other words,

NOTE Confidence: 0.78377306

00:10:06.008 --> 00:10:08.264 if you allocated 50 microliters of

NOTE Confidence: 0.78377306

00:10:08.264 --> 00:10:10.458 control cells to the control tubes,

NOTE Confidence: 0.78377306

00:10:10.460 --> 00:10:12.445 the volume of staining antibody

NOTE Confidence: 0.78377306

00:10:12.445 --> 00:10:14.430 cannot exceed 50 microliters so

NOTE Confidence: 0.78377306

00:10:14.494 --> 00:10:16.659 that the total staining volume

NOTE Confidence: 0.78377306

00:10:16.659 --> 00:10:17.958 remains 100 microliters.

NOTE Confidence: 0.78377306

00:10:17.960 --> 00:10:19.808 If the volume of the staining

NOTE Confidence: 0.78377306

00:10:19.808 --> 00:10:21.970 antibodies is less than 50 microliters,

NOTE Confidence: 0.78377306

00:10:21.970 --> 00:10:23.968 then add additional facts buffer to

NOTE Confidence: 0.78377306

00:10:23.968 --> 00:10:26.051 bring the final staining volume to

NOTE Confidence: 0.78377306

00:10:26.051 --> 00:10:28.115 100 microliters for control tubes and

NOTE Confidence: 0.78377306

00:10:28.115 --> 00:10:29.979 one milliliter for the sample tube.

NOTE Confidence: 0.80317324

00:10:33.140 --> 00:10:35.798 Once all cells Facs buffer an,

NOTE Confidence: 0.80317324

00:10:35.800 --> 00:10:38.894 antibodies are added to the staining tubes,

NOTE Confidence: 0.80317324

00:10:38.900 --> 00:10:41.224 incubate according to the

NOTE Confidence: 0.80317324

00:10:41.224 --> 00:10:42.386 manufacturer's recommendations.

NOTE Confidence: 0.80317324

00:10:42.390 --> 00:10:43.842 Most antibodies require incubation

NOTE Confidence: 0.80317324

00:10:43.842 --> 00:10:46.640 for 30 minutes on ice in the dark.

NOTE Confidence: 0.83693075

00:10:49.390 --> 00:10:51.406 After the primary stain is complete,

NOTE Confidence: 0.83693075

00:10:51.410 --> 00:10:53.492 add one milliliter of fax buffer

NOTE Confidence: 0.83693075

00:10:53.492 --> 00:10:55.636 to the control tubes and two

NOTE Confidence: 0.83693075

00:10:55.636 --> 00:10:57.682 milliliters of fax buffer to the

NOTE Confidence: 0.83693075

00:10:57.682 --> 00:10:59.807 sample tube and spin at 1200 RPM,

NOTE Confidence: 0.83693075

00:10:59.810 --> 00:11:01.820 4 degrees Celsius for 10 minutes.

NOTE Confidence: 0.8434153

00:11:06.140 --> 00:11:07.610 When the spin is complete,

NOTE Confidence: 0.8434153

00:11:07.610 --> 00:11:08.870 discard the supernatant, taking

NOTE Confidence: 0.8434153

00:11:08.870 --> 00:11:11.071 great care not to discard the cell

NOTE Confidence: 0.8434153

00:11:11.071 --> 00:11:12.877 pellets at the bottom of the tubes.

NOTE Confidence: 0.7804666

00:11:21.790 --> 00:11:24.086 If any of the antibodies used in the

NOTE Confidence: 0.7804666

00:11:24.086 --> 00:11:26.279 first stain were conjugated to biotin,

NOTE Confidence: 0.7804666

00:11:26.280 --> 00:11:27.828 a secondary stain with

NOTE Confidence: 0.7804666

00:11:27.828 --> 00:11:29.376 streptavidin conjugated to a

NOTE Confidence: 0.7804666

00:11:29.376 --> 00:11:31.367 unique floor 4 will be required.

NOTE Confidence: 0.7804666

00:11:31.370 --> 00:11:33.260 Similarly to the primary stain,

NOTE Confidence: 0.7804666

00:11:33.260 --> 00:11:35.435 add the appropriate amount of

NOTE Confidence: 0.7804666

00:11:35.435 --> 00:11:37.175 streptavidin according to the

NOTE Confidence: 0.7804666

00:11:37.175 --> 00:11:39.250 titration to the appropriate tubes.

NOTE Confidence: 0.7804666

00:11:39.250 --> 00:11:41.714 You don't have to add this to single

NOTE Confidence: 0.7804666

00:11:41.714 --> 00:11:43.900 color control tubes that were not

NOTE Confidence: 0.7804666

00:11:43.900 --> 00:11:45.780 stained with a biotin conjugated

NOTE Confidence: 0.7804666

00:11:45.780 --> 00:11:48.588 antibody or to an FMO tube in which the

NOTE Confidence: 0.7804666

00:11:48.588 --> 00:11:51.358 biotin conjugated antibody was omitted.

NOTE Confidence: 0.7804666

00:11:51.360 --> 00:11:53.405 Bring the total staining volume

NOTE Confidence: 0.7804666

00:11:53.405 --> 00:11:55.937 up to 100 microliters for control

NOTE Confidence: 0.7804666

00:11:55.937 --> 00:11:58.391 teams or one milliliter for sample

NOTE Confidence: 0.7804666

00:11:58.391 --> 00:12:00.200 tubes with fax buffer.

NOTE Confidence: 0.7804666

00:12:00.200 --> 00:12:02.452 Incubate according to the

NOTE Confidence: 0.7804666

00:12:02.452 --> 00:12:03.578 manufacturer's recommendation.

NOTE Confidence: 0.7804666

00:12:03.580 --> 00:12:04.892 Most antibodies require incubation

NOTE Confidence: 0.7804666

00:12:04.892 --> 00:12:07.419 for 30 minutes on ice in the dark.

NOTE Confidence: 0.8323322

00:12:11.860 --> 00:12:13.540 Once secondary staining is complete,

NOTE Confidence: 0.8323322

00:12:13.540 --> 00:12:16.074 repeat the wash and spin steps as

NOTE Confidence: 0.8323322

00:12:16.074 --> 00:12:18.600 was done after the primary stain.

NOTE Confidence: 0.8323322

00:12:18.600 --> 00:12:20.570 Resuspend the control pellets in

NOTE Confidence: 0.8323322

00:12:20.570 --> 00:12:22.540 200 microliters of Facs buffer

NOTE Confidence: 0.8323322

00:12:22.608 --> 00:12:24.423 and 300 microliters of Facs

NOTE Confidence: 0.8323322

00:12:24.423 --> 00:12:26.238 buffer for the sample palette.

NOTE Confidence: 0.8323322

00:12:26.240 --> 00:12:27.860 Filter these cell suspensions through

NOTE Confidence: 0.8323322

00:12:27.860 --> 00:12:30.274 a 40 or 100 Micron filter depending

NOTE Confidence: 0.8323322

00:12:30.274 --> 00:12:32.464 on the expected size of yourselves

NOTE Confidence: 0.8323322

00:12:32.464 --> 00:12:34.238 and proceed to the cytometer.

NOTE Confidence: 0.78835535

00:12:40.540 --> 00:12:42.400 Prime just sorting the cells.

NOTE Confidence: 0.78835535

00:12:42.400 --> 00:12:44.255 Prepare collection tubes or plates

NOTE Confidence: 0.78835535

00:12:44.255 --> 00:12:46.110 to receive the sorted cells.

NOTE Confidence: 0.78835535

00:12:46.110 --> 00:12:48.138 Confirmed that Uber plate you wish

NOTE Confidence: 0.78835535

00:12:48.138 --> 00:12:49.971 to use fits the specification

NOTE Confidence: 0.78835535

00:12:49.971 --> 00:12:52.407 of the cytometer to be used.

NOTE Confidence: 0.78835535

00:12:52.410 --> 00:12:54.254 Typically, growth media supplemented

NOTE Confidence: 0.78835535

00:12:54.254 --> 00:12:57.020 with survival cytokines can be used

NOTE Confidence: 0.78835535

00:12:57.087 --> 00:12:59.235 to collect certain cells into and

NOTE Confidence: 0.78835535

00:12:59.235 --> 00:13:01.699 results in better post sort viability.

NOTE Confidence: 0.78835535

00:13:01.700 --> 00:13:02.600 Of special note,

NOTE Confidence: 0.78835535

00:13:02.600 --> 00:13:04.700 be sure to follow all safety policies

NOTE Confidence: 0.78835535

00:13:04.763 --> 00:13:06.459 and procedures when transporting

NOTE Confidence: 0.78835535

00:13:06.459 --> 00:13:08.155 yourselves to the cytometer.

NOTE Confidence: 0.78835535

00:13:08.160 --> 00:13:08.912 For example,

NOTE Confidence: 0.78835535

00:13:08.912 --> 00:13:11.168 unfixed human CD 34 positive cells

NOTE Confidence: 0.78835535

00:13:11.168 --> 00:13:13.083 are required to be transported

NOTE Confidence: 0.78835535

00:13:13.083 --> 00:13:15.267 in a secondary container that is

NOTE Confidence: 0.78835535

00:13:15.267 --> 00:13:17.136 labeled with a BSL two sticker.

NOTE Confidence: 0.7975483

00:13:19.290 --> 00:13:20.834 Once at the cytometer,

NOTE Confidence: 0.7975483

00:13:20.834 --> 00:13:22.764 set the color parameters that

NOTE Confidence: 0.7975483

00:13:22.764 --> 00:13:24.990 will be used to sort the cells.

NOTE Confidence: 0.7975483

00:13:24.990 --> 00:13:26.830 Create compensation tubes for each

NOTE Confidence: 0.7975483

00:13:26.830 --> 00:13:29.064 single color control tube you have

NOTE Confidence: 0.7975483

00:13:29.064 --> 00:13:31.038 and create a new specimen entitled,

NOTE Confidence: 0.7975483

00:13:31.040 --> 00:13:35.144 FMO and generate tubes for each FML you have.

NOTE Confidence: 0.7975483

00:13:35.150 --> 00:13:38.776 Also create a tube for your sample.

NOTE Confidence: 0.7975483

00:13:38.780 --> 00:13:40.610 Using the unstained control cells,

NOTE Confidence: 0.7975483

00:13:40.610 --> 00:13:42.806 adjust the voltages for each channel,

NOTE Confidence: 0.7975483

00:13:42.810 --> 00:13:44.254 including FC and SC,

NOTE Confidence: 0.7975483

00:13:44.254 --> 00:13:46.819 so that your cells are mostly clustered

NOTE Confidence: 0.7975483

00:13:46.819 --> 00:13:49.744 in the center of the FC SC dot plot

NOTE Confidence: 0.7975483

00:13:49.817 --> 00:13:52.199 and the background signal in each

NOTE Confidence: 0.7975483

00:13:52.199 --> 00:13:55.770 color channel is well below 2:50.

NOTE Confidence: 0.7975483

00:13:55.770 --> 00:13:56.382 Before recording,

NOTE Confidence: 0.7975483

00:13:56.382 --> 00:13:58.218 check those voltages on each of

NOTE Confidence: 0.7975483

00:13:58.218 --> 00:13:59.680 the single color control tubes

NOTE Confidence: 0.7975483

00:13:59.680 --> 00:14:01.276 as well as the sample tube.

NOTE Confidence: 0.7975483

00:14:01.280 --> 00:14:02.960 Adjust voltages for each channel

NOTE Confidence: 0.7975483

00:14:02.960 --> 00:14:05.234 so that the negative peak is below

NOTE Confidence: 0.7975483

00:14:05.234 --> 00:14:07.096 10 to the third and the positive

NOTE Confidence: 0.7975483

00:14:07.096 --> 00:14:08.820 peak is below 10 to the five.

NOTE Confidence: 0.879604

00:14:10.870 --> 00:14:13.397 Once you are satisfied with the voltages,

NOTE Confidence: 0.879604

00:14:13.400 --> 00:14:15.210 begin by recording single color

NOTE Confidence: 0.879604

00:14:15.210 --> 00:14:17.020 controls and calculate the compensation.

NOTE Confidence: 0.879604

00:14:17.020 --> 00:14:20.200 Once all have been recorded.

NOTE Confidence: 0.879604

00:14:20.200 --> 00:14:21.532 Then record the FML's.

NOTE Confidence: 0.879604

00:14:21.532 --> 00:14:23.530 Use the recorded data from the

NOTE Confidence: 0.879604

00:14:23.594 --> 00:14:25.514 Fmo's to help draw and position

NOTE Confidence: 0.879604

00:14:25.514 --> 00:14:27.490 gates based on true negatives.

NOTE Confidence: 0.8112558

00:14:29.960 --> 00:14:31.927 Once all of the gates of the

NOTE Confidence: 0.8112558

00:14:31.927 --> 00:14:33.449 sorting strategy have been drawn,

NOTE Confidence: 0.8112558

00:14:33.450 --> 00:14:35.935 load the sample onto the cytometer and

NOTE Confidence: 0.8112558

00:14:35.935 --> 00:14:38.129 set the population you wish to sort.

NOTE Confidence: 0.8112558

00:14:38.130 --> 00:14:40.105 Also specify what kind of

NOTE Confidence: 0.8112558

00:14:40.105 --> 00:14:41.290 collection container you're

NOTE Confidence: 0.8112558

00:14:41.290 --> 00:14:43.587 using and how many cells to sort.

NOTE Confidence: 0.8112558

00:14:43.590 --> 00:14:45.155 Then begin sorting your sample

NOTE Confidence: 0.8112558

00:14:45.155 --> 00:14:46.720 tube and collecting your enriched

NOTE Confidence: 0.8112558

00:14:46.777 --> 00:14:48.689 population for downstream applications.

NOTE Confidence: 0.8187878

00:15:00.820 --> 00:15:03.262 After the service complete, use the

NOTE Confidence: 0.8187878

00:15:03.262 --> 00:15:05.760 cells for your downstream application.

NOTE Confidence: 0.8187878

00:15:05.760 --> 00:15:08.748 If sorted cells were collected in a fax tube,

NOTE Confidence: 0.8187878

00:15:08.750 --> 00:15:10.969 add 2 milliliters of I MDM media

NOTE Confidence: 0.8187878

00:15:10.969 --> 00:15:13.354 to wash the cells in the sorted

NOTE Confidence: 0.8187878

00:15:13.354 --> 00:15:15.388 tube and spent at 1200 RPM,

NOTE Confidence: 0.8187878

00:15:15.390 --> 00:15:17.376 4 degrees Celsius for 10 minutes.

NOTE Confidence: 0.77992374

00:15:20.680 --> 00:15:22.750 Resuspend the cell pellet in the

NOTE Confidence: 0.77992374

00:15:22.750 --> 00:15:25.050 appropriate growth media or freezing media.

NOTE Confidence: 0.75087214

00:15:28.480 --> 00:15:30.905 This concludes the protocol for

NOTE Confidence: 0.75087214

00:15:30.905 --> 00:15:33.650 Multicolor Facs sorting of CD 34.

NOTE Confidence: 0.75087214

00:15:33.650 --> 00:15:36.470 Positive amount Aquatic Center gender cells.