Product Information

ViaPlex™ 2-Color Cell Barcoding Kit (405 & 488)

Kit Contents

Component	30148-T 3 assays[1]	30148 10 assays[2]
ViaPlex™ 405 Cell Barcoding Dye	30148A 3 vials	30148A 10 vials
ViaPlex™ 488 Cell Barcoding Dye	30148B 3 vials	30148B 10 vials
DMSO, Anhydrous	99938 1 x 500 uL	99938 3 x 500 uL

[1] 30148-T contains sufficient dye to perform three 15-spot assays, or 45 total cell samples.

[2] 30148 contains sufficient dye to perform ten 15-spot assays, or 150 total cell samples.

Note: One assay can barcode up to 15* different cell populations (1 x 10^6 cells each), which can then be combined to generate up to 15 flow cytometry samples.

*16 different cell populations can be barcoded with additional compensation. See “Assay Considerations” for more information.

Storage and Handling

Each ViaPlex™ Dye is packaged in a moisture barrier foil bag with a desiccant pack. Store unopened dye vial bags at -20°C. Anhydrous DMSO may be stored at room temperature, 4°C, or -20°C. Product is stable for at least 6 months from date of receipt when stored as recommended.

ViaPlex™ Dyes are provided as a lyophilized solid. Stock solutions should be prepared using the provided anhydrous DMSO on the day of use. If performing the full 15-sample assay, each dye vial will be single use. If fewer samples are being tested and there is dye stock solution remaining, the reconstituted ViaPlex™ Dye stock vial may be stored tightly sealed with desiccant, protected from light, at -20°C. Lower concentration dye dilutions in DMSO should be prepared fresh on the day of use (see protocol).

Spectral Properties

ViaPlex™ 405 Ex/Em: 387/446 nm
ViaPlex™ 488 Ex/Em: 495/524 nm

Product Description

The ViaPlex™ 2-Color Cell Barcoding Kit (405 & 488) is designed for fluorescent cell barcoding for flow cytometry. Using combinatorial fluorescent cell staining, the ViaPlex™ kit allows up to 15 distinct cell populations to be combined and assayed together in one tube by flow cytometry. Cell barcoding reduces the amount of reagents needed to stain the cells and the time needed to measure a large number of samples, by combining them into one staining reaction. Thus, fluorescent cell barcoding saves cost and time when screening cells by flow cytometry.

The ViaPlex™ 2-Color Barcoding Kit (405 & 488) uses two reactive fluorescent dyes, ViaPlex™ 405 Barcoding Dye and ViaPlex™ 488 Barcoding Dye. Each dye is cell permeant, and covalently and stably binds proteins in the cytoplasm of cells. This protocol contains instructions for staining cells with combinations of the two dyes in a 15-plex matrix (or 16-plex with additional compensation). After washing, the stained cells can then be combined, allowing up to 15 distinct cell populations to be gated and deconvoluted during flow analysis.

The ViaPlex™ kit is highly flexible for different workflows. Because the barcoding is performed on live cells, it is compatible with both surface and intracellular antibody staining, allowing a wide variety of staining experiments to be performed. Barcoding can also generally be performed either before or after cell treatment, for added experimental flexibility. Unlike other barcoding methods, ViaPlex™ labeling does not require cell fixation or permeabilization, allowing assays to be done on live cells. However, because the labeling is covalent, cells can be fixed and permeabilized after barcoding, which also permits intracellular labeling.

Assay Considerations

- The protocols below are for one 15-spot barcoding assay (or 16-spot with additional compensation). One vial of each dye is sufficient to barcode 16 cell samples (1 x 10^6 cells/mL each). After combining barcoded samples, we recommend using at least 500 uL of 1 x 10^6 cells (2 x 10^6 cells/mL) per sample for flow cytometry.
- You can test up to 15 cell samples in one barcoding assay. To test more cell samples, you can perform multiple barcode assays with up to 15 cell samples in each assay.
- A 16th barcode (405H) can be added if desired but will require compensation for clear separation (see Figure 2).
- To scale up for more samples, increase the number of dye vials (one vial for up to 15 samples, two vials for up to 30 samples, etc). Reconstitute each dye in DMSO as described in the protocol below but combine all of the high (H) stocks into a single tube and scale up the volumes for the medium (M) and low (L) stocks proportionally.

Assay Considerations (continued from page 1)

- We do not recommend barcoding adherent cells while on a plate, but adherent cells can be barcoded in solution after dissociation. Attached cells show less homogeneous labeling than cells in suspension, resulting in broader barcode populations. However, if barcoding adhered cells is desired, we suggest using fewer total barcodes (e.g., 6 instead of 15).
- For large or heterogeneous cells, or adherent cells stained on a plate, we recommend using only the following 6 barcodes in order to maintain spacing between the populations: Unstained, 405M, 488H, 405L488M, 405H488M, and 405H488H.

Experimental Protocols

Materials required but not provided

- Buffer without amines or proteins (i.e., PBS or HBSS)
- FACS buffer containing serum or other protein-containing blocking agent
- Fixation buffer (if needed for intracellular staining)
- Permeabilization buffer (if needed for intracellular staining)
- Antibodies or other stains of interest with dye colors complementary to ViaPlex™ 405 and ViaPlex™ 488 (see Spectral Properties)

1. Cell preparation

1.1 For this procedure, we suggest plating cells in a 24-well plate with 1 x 10^6 cells per well. Plate up to 15 wells for one barcoding assay. To test more than 15 samples, see "Assay Considerations".

1.2 Treat the cells as desired (e.g., add any drugs or cytokines for the desired amount of time).

Notes:

a. You may perform the fluorescent barcoding prior to cell treatment if desired, because the barcoding dyes are nontoxic. This is only advisable if the cell treatment time is less than 6 hours, otherwise the barcoding dyes may be diluted by cell division.

b. Cell barcoding must be performed before staining with antibodies.

Table 1. ViaPlex™ Dye Stock Solutions

ViaPlex™ Dilution Tube	Volume of DMSO	Volume of ViaPlex™ Dye
405H	10 uL	Add DMSO to ViaPlex™ 405 dye vial (Cat. No. 30148A)
405M	18 uL	2 uL of 405H
405L	18 uL	2 uL of 405M
488H	12 uL	Add DMSO to ViaPlex™ 488 dye vial (Cat. No. 30148B)
488M	48 uL	2 uL of 488H
488L	23 uL	2 uL of 488M

2. Barcode dye preparation

You will prepare a dilution series of each dye so that you end up with high, medium, and low concentrations of each dye, and then prepare a matrix of dye combinations. Each dye has a different dilution scheme. See Table 1, Table 2, and detailed instructions below.

Note: Dilutions should be prepared fresh immediately before barcode staining.

2.1 Retrieve one vial of each ViaPlex™ Barcoding Dye component (Cat. No. 30148A, 30148B) and one vial of anhydrous DMSO (Cat. No. 99938).

2.2 Prepare a dilution series of three concentrations of ViaPlex™ 405 Dye (high (H), medium (M), and low (L)) (see Table 1):

a. Obtain two clean microcentrifuge tubes. Label one "405M" and one "405L".

b. Add 18 uL of anhydrous DMSO to both empty tubes.

c. For 405H: Dissolve one vial of ViaPlex™ 405 Cell Barcoding Dye (Cat. No. 30148A) in 10 uL anhydrous DMSO. Pipette up and down and vortex to mix well. Briefly centrifuge the vial to gather the liquid in the bottom of the tube. Label this tube "405H".

d. For 405M: Add 2 uL of the "405H" dye solution to the DMSO in the "405M" tube. Pipette up and down to mix well. Briefly centrifuge the vial to gather the liquid in the bottom of the tube.

e. For 405L: Add 2 uL of the "405M" dye solution to the DMSO in the "405L" tube. Pipette up and down to mix well. Briefly centrifuge the vial to gather the liquid in the bottom of the tube.

2.3 Prepare a dilution series of three concentrations of ViaPlex™ 488 Dye (high (H), medium (M), and low (L)) (see Table 1):

a. Obtain two clean microcentrifuge tubes. Label one "488M" and one "488L".

b. Add 48 uL of anhydrous DMSO to the "488M" tube.

c. Add 23 uL of anhydrous DMSO to the "488L" tube.

d. For 488H: Dissolve one vial of ViaPlex™ 488 Cell Barcoding Dye (Cat. No. 30148B) in 12 uL anhydrous DMSO. Pipette up and down and vortex to mix well. Briefly centrifuge the vial to gather the liquid in the bottom of the tube. Label this tube "488H".

e. For 488M: Add 2 uL of the "488H" dye solution to the DMSO in the "488M" tube. Pipette up and down to mix well. Briefly centrifuge the vial to gather the liquid in the bottom of the tube.

f. For 488L: Add 2 uL of the "488M" dye solution to the DMSO in the "488L" tube. Pipette up and down to mix well. Briefly centrifuge the vial to gather the liquid in the bottom of the tube.

Table 2. ViaPlex™ Dye Combination Matrix

Dye Stock	No 405	405L	405M	405H
No 488	**1) No dye** 4 uL DMSO	**2) 405L only** 2 uL 405L 2 uL DMSO	**3) 405M only** 2 uL 405M 2 uL DMSO	Not recommended*
488L	**4) 488L only** 2 uL 488L 2 uL DMSO	**5) 405L488L** 2 uL 405L 2 uL 488L	**6) 405M488L** 2 uL 405M 2 uL 488L	**7) 405H488L** 2 uL 405H 2 uL 488L
488M	**8) 488M only** 2 uL 488M 2 uL DMSO	**9) 405L488M** 2 uL 405L 2 uL 488M	**10) 405M488M** 2 uL 405M 2 uL 488M	**11) 405H488M** 2 uL 405H 2 uL 488M
488H	**12) 488H only** 2 uL 488H 2 uL DMSO	**13) 405L488H** 2 uL 405L 2 uL 488H	**14) 405M488H** 2 uL 405M 2 uL 488H	**15) 405H488H** 2 uL 405H 2 uL 488H

*See Assay Considerations and section 5 for more information.

2.4 Prepare a 15-plex matrix of dye combinations as follows (see Table 2 above).

Note: A 16th "405H only" is not recommended due to bleed-through into the FITC detection channel. However, if a 16th sample is desired, compensation may be used to correct for the bleed-through.

a. Obtain 15 clean microcentrifuge tubes, or a deep-well microplate, depending on which you plan to use to stain your cells (see sections 3 and 4). If using tubes, label them 1-15.

b. Add 2 uL of DMSO and/or dye to each tube or well as shown in Table 2 on page 3. Each tube will have 4 uL total added to it. Tube 1, with no barcode dye, will have 4 uL of DMSO. If using a microplate, set up the wells according to Table 2 above.

3. Barcoding suspension cells in tubes

Below is the protocol for barcoding suspension cells in tubes. For barcoding suspension cells in plates, see section 4.

Note: This protocol describes how to perform one 15-spot barcode assay. See "Assay Considerations" if you plan to perform more than one barcoding assay.

3.1 Start with 15 cell samples in individual tubes labeled 1-15, prepared and treated as desired. Each sample should contain at least 1 x 10^6 cells in 1 mL.

Note: Using 1 mL of 1 x 10^6 cells/mL for each cell population is sufficient for 15 samples for flow cytometry. If you set aside some of the 405H488H and No dye individual cell samples, they can be used to adjust the instrument detector gains as in step 5.2 and you can have up to 14 samples for flow cytometry.

3.2 Pellet the cells by centrifugation and resuspend in 1 mL of buffer (PBS or HBSS).

3.3 One by one, transfer the cells for from each numbered tube into the dye-containing tube of the same number prepared in section 2. Immediately mix by pipetting up and down.

Note: Work quickly during this step to avoid differences in staining time between samples.

3.4 Incubate the cells in dye for 15 minutes at 37°C, protected from light.

3.5 Pellet the cells and resuspend in 1 mL of complete culture medium (with serum if applicable for your cell type) (or serum-containing FACS buffer).

3.6 Incubate the cells for 5 additional minutes at 37°C.

Note: This step is required to inactivate excess free dye.

3.7 Pellet the cells and resuspend in 1 mL of FACS buffer to wash the cells.

3.8 Repeat step 3.7 two additional times for three washes total.

3.9 Resuspend the cells in 500 uL FACS buffer (2 x 10^6 cells/mL).

3.10 Combine equal volumes of each of the 15 barcoded cell populations into a single tube. The volume will depend on how many staining reactions you plan to do on the barcoded cell mixture.

Example: If your assay is to stain with a single test antibody along with an unstained control and an isotype control (i.e., three total flow samples), add 100 uL of each barcoded cell sample into a single tube to pool 1.5 mL cells total at 2 x 10^6 cells/mL. Scale accordingly for more FACS tubes.

Optional: Set aside some of the 405H488H and No dye individual cell samples to adjust instrument detector gains.

3.11 Aliquot 500 uL (1 x 10^6) of the combined cells per FACS tube for staining your targets of interest. Scale as needed if more stains are to be performed.

3.12 Stain the cells for your targets of interest. Use antibodies or probes with dye colors complementary to ViaPlex™ 405 and ViaPlex™ 488.

a. To perform cell surface staining, pellet the cells and stain with antibodies or other probes.

b. To perform intracellular staining, pellet the cells and proceed with your desired fixation and permeabilization methods, followed by staining with antibodies or other probes.

3.13 Proceed to detection, gating, and analysis (section 5).

4. Barcoding suspension cells in plates

4.1 Start with 15 cell samples, prepared and treated as desired. Each sample should contain at least 1 x 10^6 cells in 1 mL.

Note: Using 1 mL of 1 x 10^6 cells/mL for each cell population is sufficient for 15 samples for flow cytometry. If you set aside some of the 405H488H and No dye individual cell samples, they can be used to adjust the instrument detector gains as in step 5.2 and you can have up to 14 samples for flow cytometry.

4.2 Transfer 1 x 10^6 cells from each cell sample into a fresh deep-well microplate, using the same layout that you used to set up your dye matrix in section 2.

4.3 Centrifuge the plate and dump off the residual liquid.

4.4 Add 1 mL of buffer (PBS or HBSS) to each well and vortex gently to resuspend the cells.

4.5 Either one by one or with a multichannel pipette, transfer the cells from the cell-containing plate to the dye-containing plate. Immediately mix by pipetting up and down.

Note: If pipetting each well individually, work quickly during this step to avoid differences in staining time between wells.

4.6 Incubate the cells in dye for 15 minutes at 37°C, protected from light.

4.7 Centrifuge the plate, remove the residual liquid, and add 1 mL of complete culture medium (with serum if applicable for your cell type) (or serum-containing FACS buffer). Vortex gently to mix.

4.8 Incubate the cells for 5 additional minutes at 37°C.

Note: This step is required to inactivate excess free dye.

4.9 Centrifuge the plate, remove residual liquid, and add FACS buffer to wash the cells. Perform two additional washes in FACS buffer, for three washes total.

4.10 Resuspend the cells in 500 uL FACS buffer (2 x 10^6 cells/mL).

4.11 Combine equal volumes of each of the 15 barcoded cell populations into a single tube. The volume will depend on how many staining reactions you plan to do on the barcoded cell mixture.

Example: If your assay is to stain with a single test antibody along with an unstained control and an isotype control (i.e., three total flow samples), add 100 uL of each barcoded cell sample into a single tube to pool 1.5 mL cells total at 2 x 10^6 cells/mL. Scale accordingly for more FACS tubes.

Optional: Set aside some of the 405H488H and No dye individual cell samples to adjust instrument detector gains.

4.12 Aliquot 500 uL (1 x 10^6) of the combined cells per FACS tube for staining your targets of interest. Scale as needed if more stains are to be performed.

4.13 Stain the cells for your targets of interest. Use antibodies or probes with dye colors complementary to ViaPlex™ 405 and ViaPlex™ 488.

a. To perform cell surface staining, pellet the cells and stain with antibodies or other probes.

b. To perform intracellular staining, pellet the cells and proceed with your desired fixation and permeabilization methods, followed by staining with antibodies or other probes.

4.14 Proceed to detection, gating, and analysis (section 5).

5. Flow cytometry detection, gating, and analysis

Note: If a 16th barcode (405H) was included, use the individual 405H and 488H cell samples to set up a compensation matrix on your flow cytometer prior to running the combined barcode sample (see Figure 2).

5.1 Gate on your cells of interest as usual (e.g., single cells, live cells, lymphocytes, etc.).

5.2 Set up a dot plot with a channel for detecting each ViaPlex™ Barcode Dye on one of the axes (see Spectral Properties for the dye Ex/Em; see the product page for recommended laser and filter sets; see Figure 1 for example dot plot). If you set aside some of the 405H488H and No dye individual cell samples, they can be used to adjust the instrument detector gains and the dot plot display to ensure that all 15 of the barcodes are well-separated and falling within the plot area.

5.3 Set gates on each of the 15 individual barcoded populations, numbering them as you did in the experiment. See Figure 1 for an example.

5.4 Since you will be analyzing 15 different populations within the same samples, ensure that you collect enough cell events to get meaningful data for each population. For homogenous cultured cells, 60,000 total cell events is enough to collect ~3000 events per barcode. However, for rarer populations such as sub-populations of PBMCs, you should collect as many cell events as possible (e.g., 1 x 10^6 cells) in order to get good data for each barcode.

5.5 Detect your antibodies or other stains of interest in the appropriate channels, analyzing each of the 15 barcoded samples separately, or overlaid to compare them.

Troubleshooting

Problem	Solutions
The 15 barcode spots are not well spaced on the 405/488 dot plot.	You can try adjusting instrument gains, laser power, or axis display settings to ensure that each spot is visible in the plot and well separated from each other. Some fixation and permeabilization conditions may compress the spots on the display. **Note:** Additional compensation is required if using 16 barcodes (see note to step 5).
Poor staining with a ViaPlex™ Dye.	• Ensure that the dye was stored lyophilized and protected from light in the foil bag. • Ensure that the dye was resuspended in anhydrous DMSO shortly before the staining was performed. • Ensure that cells were washed and stained using amine-free buffer. • Ensure that the cells were adequately mixed immediately after dye addition.

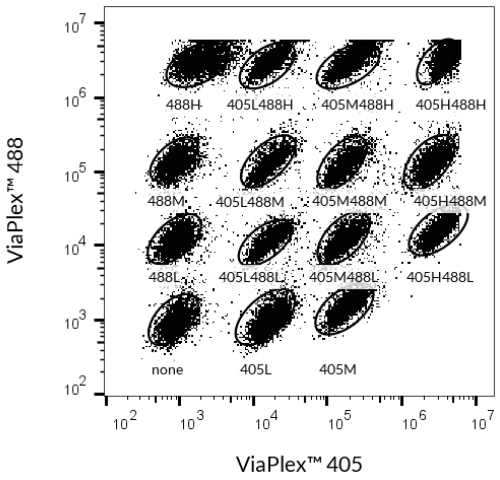

Figure 1. Example flow cytometry scatter plot from a 15-plex assay showing the segregation of each barcode population in the mixed sample.

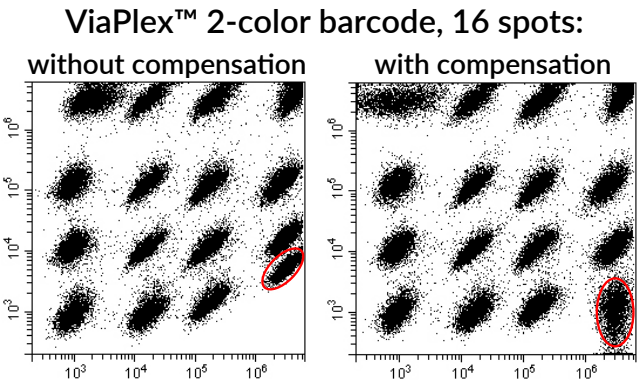

Figure 2. A 16th barcode can be included in the mixture if compensation is performed. This figure shows that the 405H population (red oval) is poorly separated from the 405H488L population without compensation (left), but can be distinguished if compensation is performed (right).

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70054...70082	MitoView™ Mitochondrial Dyes
29001...29126	Annexin V Conjugates
30030...30073	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate & Annexin V
92230...92584	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
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