Image-iT™ Cell Painting Kit

Catalog Numbers I65000 and I65500

Pub. No. MAN0029142 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ Image-iT™ Cell Painting Kit is a collection of reagents for monitoring cell health and cytotoxicity. The kit "paints" different cellular components with small-molecule fluorescent dyes that when combined with a high-content imager, such as the CellInsight™ CX7 LZR Pro High Content Screening (HCS) Platform, provides a phenotypic readout of cell status. Individual dye components can be used directly from a 100X stock solution or titrated to optimize staining for unique combinations of cell types, plating conditions, and/or microscope configuration.

Contents and storage

The Image-iT™ Cell Painting Kit contains sufficient reagents to stain 2 × 96-well plates (Cat. No. I65000) or 10 × 96-well plates (Cat. No. I65500).

Item	Cellular target	Cat. No. I65000	Cat. No. I65500	Working concentration	Storage
Hoechst 34580	DNA, nucleus	110 µg	5 × 110 µg	1–5 µg/mL	–20°C
Concanavalin A, Alexa Fluor™ 488 Conjugate	Glycoproteins, endoplasmic reticulum	2.2 mg	5 × 2.2 mg	10–100 µg/mL	
Wheat Germ Agglutinin, Alexa Fluor™ 555 Conjugate	Plasma membrane, golgi apparatus	66 µg	5 × 66 µg	0.3–3 µg/mL	
Alexa Fluor™ 568 Phalloidin	Filamentous actin, cytoskeleton	1.2 µg	5 × 1.2 µg	5–50 ng/mL	
SYTO™ 14 Green Fluorescent Nucleic Acid Stain, 5 mM (in DMSO)	RNA, cytoplasm and nucleolus	29 µL	5 × 29 µL	2–6.5 µM	
MitoTracker™ Deep Red FM	Mitochondria	6.6 µg	5 × 6.6 µg	100–300 ng/mL	

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
DMSO, Anhydrous	D12345
HBSS, calcium, magnesium, no phenol red	14025092
Distilled Water (dH_2O)	15230162
Sodium bicarbonate, 1M buffer solution, pH 8.5[1]	J60408.AP
Bovine serum albumin (BSA)	MLS
Triton™ X-100	MLS
Paraformaldehyde (PFA)	MLS

Item	Source
CellInsight™ CX7 LZR High Content Screening (HCS) Platform	CX7A1110LZR
CellInsight™ CX7 LZR Pro High Content Screening Platform	HCSDCX7LZRPRO
37°C, 5% CO_2 incubator	MLS
Plasticware	MLS

[1] Dilute 1:10 (0.1M) with dH_2O before use.

Prepare reagent stock solutions

Note: To obtain robust staining, prepare the reagents as described. Depending on the cell type, microplate, or imaging configuration, titration downward may be required to optimize the final staining concentration.

Prepare stock solutions of the following reagents.

IMPORTANT! For best results, prepare reagent stock solutions on the day of use.

Reagent	Action	Concentration of stock solution	Final staining concentration
Hoechst 34580	1. Add 220 µL of dH_2O. 2. Vortex thoroughly to mix, then centrifuge to collect the contents.	100X	5 µg/mL
Concanavalin A, Alexa Fluor™ 488 Conjugate	1. Add 220 µL of 0.1M Sodium Bicarbonate solution 2. Vortex thoroughly to mix, then centrifuge to collect the contents.	100X	100 µg/mL
Wheat Germ Agglutinin, Alexa Fluor™ 555 Conjugate	1. Add 220 µL of dH_2O. 2. Vortex thoroughly to mix, then centrifuge to collect the contents. Centrifuge immediately before use to remove normal protein aggregates.	100X	3 µg/mL
Alexa Fluor™ 568 Phalloidin	1. Add 20 µL of DMSO. 2. Vortex thoroughly to ensure all traces of dye at the bottom of the vial are reconstituted. 3. Add 200 µL of dH_2O. 4. Vortex thoroughly to mix, then centrifuge to collect the contents.	100X	50 ng/mL
SYTO™ 14 Green Fluorescent Nucleic Acid Stain, 5 mM	1. Add 191 µL of dH_2O.	100X	6.5 µM
MitoTracker™ Deep Red FM	1. Add 20 µL of DMSO. 2. Vortex thoroughly to ensure all traces of dye at the bottom of the vial are reconstituted. 3. Add 200 µL of dH_2O. 4. Vortex thoroughly to mix, then centrifuge to collect the contents.	100X	300 ng/mL

Prepare 1X blocking solution

1. Add 1 g BSA to 100 mL of HBSS, then mix until completely dissolved.
2. Add 100 µL of Triton™ X-100 detergent, then mix thoroughly.
3. Filter through a 0.2-µm membrane.

Store at 4°C for up to 2 weeks.

Perform cell painting

1 Seed cells, then incubate overnight

1. Seed cells into a 96-well plate at a density of 2,500 cells in 80 µL of media per well.
2. Incubate for 30–60 minutes at room temperature to allow the cells to settle.
3. Place the plate into a 37°C, 5% CO_2 incubator with ~95% relative humidity, then incubate the cells overnight to allow recovery and growth.

2 Treat cells with test compounds

1. Prepare test compounds at a 10X concentration, then add 10 µL directly to each well containing cells and media.

   IMPORTANT! Do not remove the media from the wells.

2. Incubate for the desired length of time.

3 Incubate cells with MitoTracker™ staining solution

1. Prepare 10X MitoTracker™ staining solution on the day of use—Transfer 110 µL of the 100X MitoTracker™ Deep Red FM stock solution to 1 mL of complete medium.
2. Vortex thoroughly, then centrifuge to collect the contents.
3. Add 10 µL of the 10X MitoTracker™ staining solution directly to each well containing cells and media. Mix thoroughly to ensure even distribution of the dye into the cell culture medium
4. Incubate for 30 minutes at 37°C, 5% CO_2, and ~95% relative humidity.

4 Fix cells with PFA-HBSS solution

1. Prepare 12 mL of PFA-HBSS solution—Combine 6 mL of 16% PFA with 6 mL of HBSS.
2. Add 100 µL of the PFA-HBSS solution directly to each well containing cells and media (final concentration = 4% PFA).
3. Cover the plate, then incubate for 20 minutes at room temperature.

   During the incubation, proceed to prepare the 1X staining solution (next step).

STOPPING POINT *(Optional)* After the incubation, cells can be washed 2 times with 150 µL of HBSS, then covered and stored for up to 2 weeks.

5 Prepare 1X staining solution

Note: The volumes provided in the following procedure are sufficient to stain one microplate of cells. For multiple microplates, scale the volumes proportionally.

IMPORTANT! Prepare 1X staining solution on the day of use.

1. Transfer 9.5 mL of 1X blocking solution to an appropriately-sized vial.
2. Add 100 µL each of the following stock solutions for a final 10-mL volume of 1X staining solution.

   (Optional) Titrate each dye independently at this step to optimize signal-to-noise ratio.

   Centrifuge the 100X stock solutions before opening and avoid protein aggregates that can be present in wheat germ agglutinin and concanavalin A solutions.

   - 100X Hoechst 34580—100 µL
   - 100X Concanavalin A, Alexa Fluor™ 488 Conjugate—100 µL
   - 100X Wheat Germ Agglutinin, Alexa Fluor™ 555 Conjugate—100 µL
   - 100X Alexa Fluor™ 568 Phalloidin—100 µL
   - 100X SYTO™ 14 Green Fluorescent Nucleic Acid Stain, 5 mM—100 µL

6 Incubate cells with 1X staining solution, then analyze

1. Remove the PFA-HBSS solution from the wells, then wash the wells 2 times with 150 µL of HBSS, removing the last wash.
2. Add 80 µL of 1X staining solution to each well of the plate.
3. Cover the plate, then incubate for 30 minutes at room temperature.
4. Remove the 1X staining solution from the wells, then wash the wells 2 times with 150 µL of HBSS.

   (Optional) If you plan to store the plate, add 2-mM sodium azide to the final wash to prevent bacterial growth.
5. Tightly seal the plate with an adhesive cover.
6. Image and analyze the plate using the CellInsight™ CX7 LZR Pro High Content Screening Platform.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0029142 A.0

Revision	Date	Description
A.0	10 February 2023	New document for the Image-iT™ Cell Painting Kit.