UltraPlex™ Chromogenic Multiplex IHC CD4, CD8, PanCK Staining Protocol CP02-LBS Kit for BOND RX/RX^{m}

Using *Cell IDx 1-RBY on Controller 7.0

Cat #: **CP02A-010** | Protocol Version 2022.01.27A

Store Entire Kit 2-8°C

Do NOT use Sodium Azide or a phosphate-based buffer in this protocol

Contents

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

Intended Use

The UltraPlex Chromogenic Multiplex IHC Kit CP02-LBS allows for the detection of CD4, CD8, and PanCK on a single tissue with one antigen retrieval step, using different chromogen colors to distinguish each biomarker.

How the Automated Protocol Works

The primary antibody cocktail of CD4, CD8, and PanCK — each conjugated to a different hapten barcode tag — will be added to the slide. This will be followed by the addition of the first secondary antibody solution (*Cell IDx Polymer 1, containing anti-CH015-AP and anti CH014-HRP). Leica Red Chromogen will be added to develop the anti CH015-AP followed by Leica Blue Chromogen addition to develop anti CH014-HRP. An HRP-arrest step will then arrest the CH014-HRP, allowing for the addition of the second secondary antibody solution (*Cell IDx Polymer 2, containing anti CH016-HRP), which will then be developed with a Yellow Chromogen. A blue or purple Hematoxylin will be used as a counterstain at the end of the protocol.

Precautions

- **For Research Use Only**. Not for diagnostic or therapeutic use.
- Consult Federal, State, and local regulations for disposal of any potentially toxic components
- Chromogen order and combinations have been selected to provide optimal staining, and Cell IDx is developing additional panels and chromogen combinations. Consult with Cell IDx before substituting or adding any markers or chromogens.
- Mount with aqueous mounting medium or Leica CV Ultra Mounting Medium. Do **not** use xylene or alcohols.

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

Staining Protocol

UltraPlex Chromogenic Multiplex IHC Reagents for CP02A-010

Cell IDx Cat#	Description	Amount Provided
SL-038/010	*Peroxidase Block 2 (Ready to Use)	2.3 mL
SL-024/010	Concentrated primary antibody cocktail (CD4-CH014, CD8-CH015, PanCK-CH016)	63 µL
SL-025/010	Concentrated Cell IDx Polymer #1 (anti-CH015-AP, anti-CH014 HRP)	33 µL
SL-026/010	Concentrated Cell IDx Polymer #2 (anti-CH016 HRP)	16.5 µL
SL-027/010	*Cell IDx Rabbit Block (Ready to Use)	2.3 mL
SL-028/010	Antibody Diluent	7.4 mL
SL-029/010	*Cell IDx HRP-arrest solution part A (Azide in MES)	2.3 mL
SL-030/010	*Cell IDx HRP-arrest solution part B (30% H_2O_2)	23 µL
SL-035/010	Yellow Chromogen	1 mL
SL-036/010	Yellow Chromogen Buffer	5 mL
SL-037/010	Purple Hematoxylin	2.3 mL
SL-047/010	Blue Hematoxylin	2.3 mL

Required Reagents/Equipment from Leica Biosystems

This mxIHC assay requires specific materials and equipment only available from Leica Biosystems

Leica Cat#	Description
AR9640	BOND Epitope Retrieval Solution 2 – 1L (RTU)
AR9222	BOND Dewax Solution – 1L (RTU)
AR9590	BOND Wash Solution 10X Concentrate – 1 L
S21.1971	BOND Mixing Stations
S21.2001	BOND Universal Covertiles – 100 pack
DS9390	BOND Polymer Refine Red Detection Kit
OPT9049	BOND Titration Kit
DC9896	BOND Blue Chromogen
OP79193	BOND 7mL Open Containers
S21.1003.D	Reagent Tray
	BOND RX/RX^{m} Fully-Automated Research Stainer

User-Supplied Material

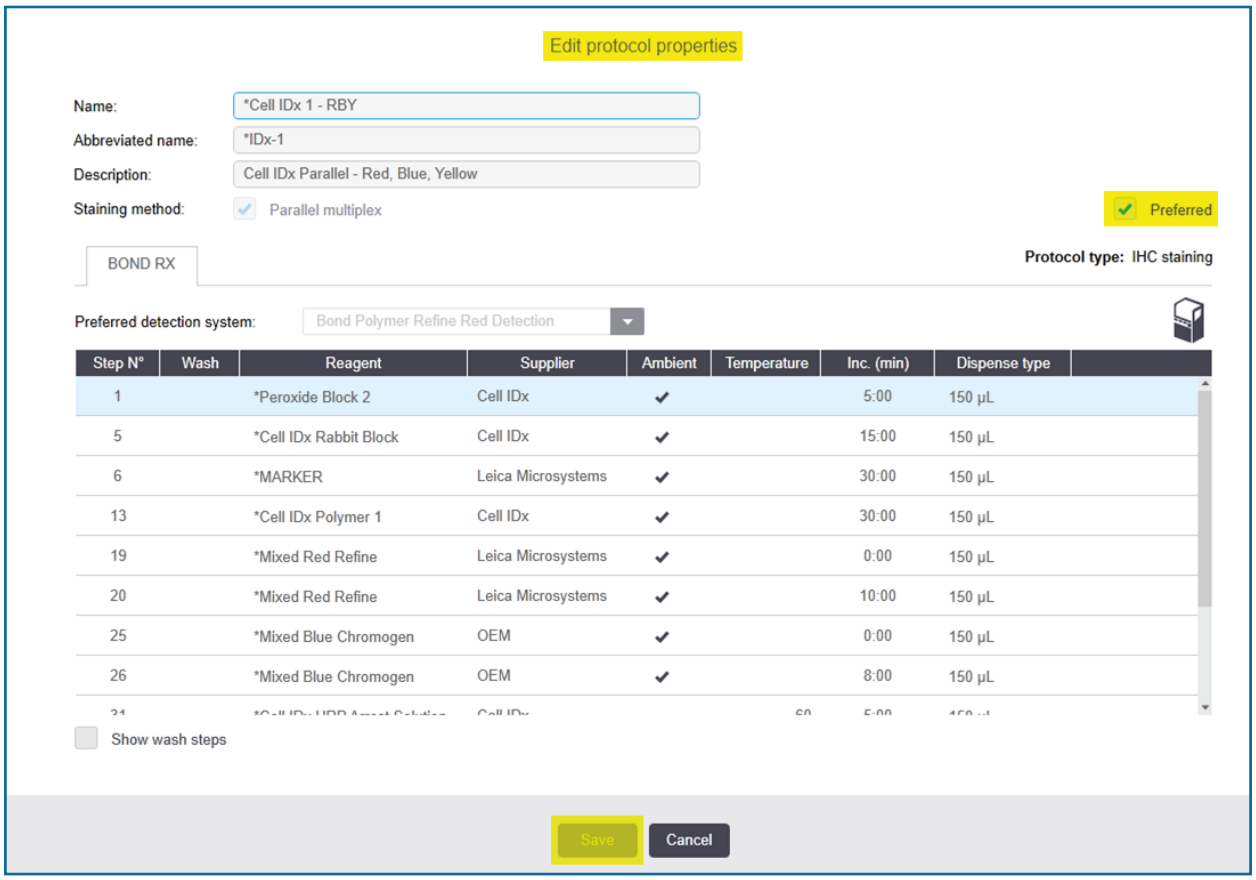
Description
Cover Glass 24 x 50mm
Deionized Water
Reagent-grade Alcohol
Aqueous Mounting Medium or Leica CV Ultra Mounting Medium

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

Change Cell IDx Protocols & Reagents to Preferred Status

Protocols

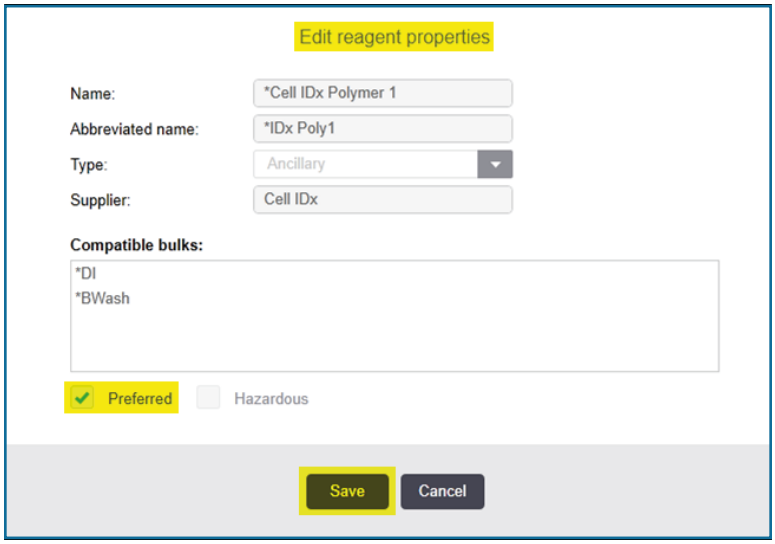
- Go to **Protocol Setup Screen** and filter by **Preferred status: All**
- Find ***Cell IDx 1-RBY**. Double-click to open the **Edit Protocol Properties** window. Make the check mark "**Preferred**" on top right of window and click **Save**.



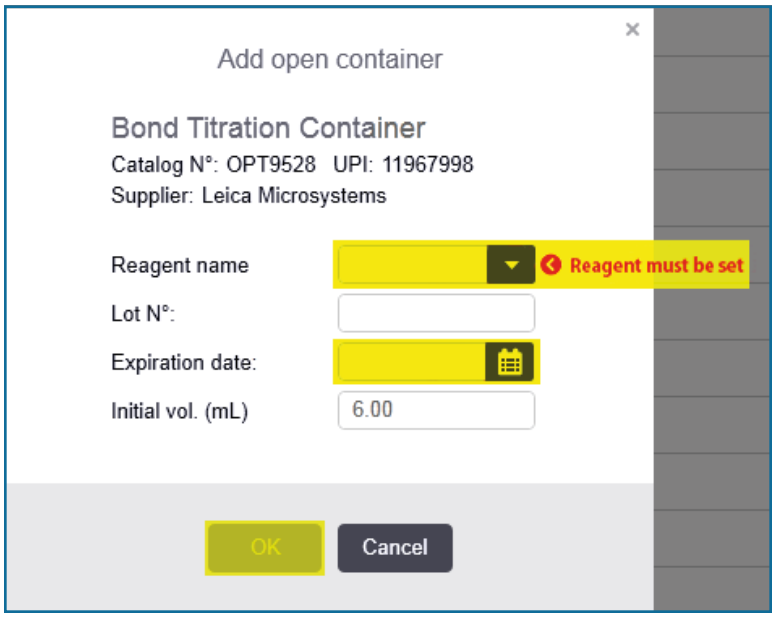
For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

Register the Reagents

- Go to **Reagent Setup Screen** and filter by **Preferred status: All**
- Find each **Cell IDx** reagent to be used (listed below):
  - *Peroxidase Block 2
  - *Cell IDx Polymer 1
  - *Cell IDx Polymer 2
  - *Cell IDx Rabbit Block
  - *Cell IDx HRP-Arrest Solution
  - *Mix 1, 20:1 Part 20A
  - *Mix 1, 20:1 Part 1B
  - *Hematoxylin
- For each one of the above, double-click to open the **Edit reagent properties** window. Make the checkmark "**Preferred**" on the bottom right of the window for each and **Save**. **Hematoxylin** should have **"Preferred"** and **"Hazardous"** checked.



- Each Cell IDx reagent will need to be registered to a **BOND Container** in order to be used on BOND RX/RX^{m}. The BOND Container can be reused/refilled until a maximum of 40 mL has been used. Once the 40 mL limit has been reached, a new BOND Container will be needed.
  - Scan the front barcode of a new 6 mL **BOND Titration Container** or 7 mL **Open Container.**

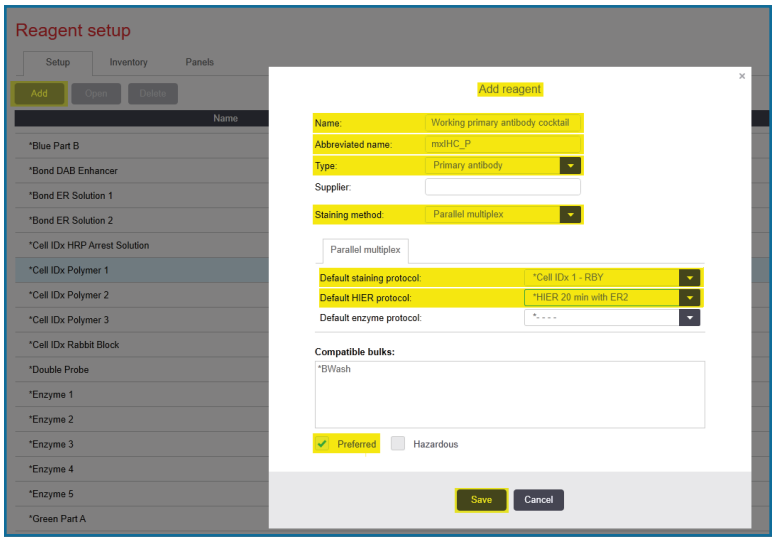


For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

- Select one of the **Cell IDx reagents** from the drop-down menu
- If required, type in a **Lot No**.
- Select an **Expiration Date** (note: *the BOND instrument will not allow you to use the container once the expiration date has passed*)
- Click on **OK**
- Ensure the **6 mL Titration Container** or **7 mL Open Container** is clearly labelled.
- Repeat for each of the **Cell IDx reagents**.
- Add the **BOND Containers** to a reagent tray.

Add Primary Antibody Reagents to System

* For the first time it is used, the working primary cocktail solution and the antibody diluent control need to be added to the database.
  - Go to **Reagent setup** screen and click on **Add**.
  - When the **Add reagent** window opens, give the reagent **a unique name and abbreviated name**. For example, "Working Primary antibody solution" as the name and "mxIHC" as the abbreviated name.
  - Assign **Type** as **Primary Antibody**
  - Assign **Staining method** as **Parallel multiplex**
  - Assign **Default staining protocol** as ***Cell IDx 1-RBY**
  - Assign **Default HIER protocol** as ***HIER 20 min with ER2**
  - Make sure to checkmark **Preferred**
  - Click on **Save**.



* Repeat the process for Adding the **Antibody Diluent Control** (to be used for the secondary antibody alone control slide) using the same settings as described above.
* Register these two reagents to a **BOND Titration Container**, as per instructions above.

Tissue Preparation

Formalin-fixed paraffin-embedded (FFPE) sections should be cut to 3 – 5 µm thickness and evenly spaced across slide surface. All tissue should be mounted on positively-charged slides for enhanced adherence. Dry/bake the slides as per your routine IHC processes for BOND RX/RX^{m}.

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

Preparation of Components Prior to Staining

Important: The BOND RX/RX^{m} mixing station allows for a maximum of six chromogens to be mixed at a given time. As a result, up to two trays (20 slides) may be run concurrently. We recommend preparing reagents for no more than two trays at a time.

Reagents provided are sufficient for one BOND run of 10 test slides and 1 slide of secondary antibody alone (no primary) control.

Note: We recommend using **6 mL BOND Titration Containers** for the **primary antibody cocktail solution**, **antibody diluent control**, ***Cell IDx Polymer 1**, ***Cell IDx Polymer 2**, ***Cell IDx HRP Arrest**, and ***Hematoxylin** since these solutions will be made fresh for each use (also to minimize dead volume).

*Peroxidase Block 2 (RTU)

This solution is RTU. Transfer 2.3 mL of ***Peroxidase Block** (**SL-038/010**) into appropriately labeled/registered 6 mL **Titration** or 7 mL **Open Container**. This can be used again for future staining runs until expiration date provided.

*Cell IDx Rabbit Block (RTU)

This solution is RTU. Transfer 2.3 mL of ***Cell IDx Rabbit Block** (**SL-027/010**) into appropriately labeled/registered 6 mL **Titration** or 7 mL **Open Container**. This can be used again for future staining runs until expiration date provided.

Working Primary Antibody Cocktail (1 to ~33 dilution)

Add 61 µL of **Concentrated Primary Antibody Cocktail** (**SL-024/010**) to appropriately labeled/registered 6 mL **Titration Container** with insert containing 1,939 µL of **Antibody Diluent** (**SL-028/010**). This solution should be made fresh for each use.

Antibody Diluent Control (for Secondary Antibody Control)

Transfer at least 500 µL of **Antibody Diluent** (**SL-028/010**) to appropriately-labeled/registered 6 mL **Titration Container** with insert.

*Cell IDx Polymer 1 Solution (anti-CH014 HRP/CH015 AP) (1 to 70 dilution)

Add 31 µL of concentrated ***Cell IDx Polymer 1** (**SL-025/010**) to appropriately labeled/registered 6 mL **Titration Container** with insert containing 2,119 µL of **Antibody Diluent** (**SL-028/10**). This solution should be made fresh for each use.

*Cell IDx Polymer 2 Solution (anti-CH016 HRP) (1 to 140 dilution)

Add 15.5 µL of concentrated ***Cell IDx Polymer 2** (**SL-026/010**) to appropriately labeled/registered 6 mL **Titration Container** with insert containing 2,134.5 µL of **Antibody Diluent** (**SL-028/010**). This solution should be made fresh for each use.

*Cell IDx HRP Arrest Solution (1 to 100 dilution)

Add 22 µL of ***Cell IDx HRP Arrest Solution Part B** (**SL-030/010**) into a 6mL **Titration Container** with insert containing 2,178 µL of **HRP Arrest Solution Part A** (**SL-029/010**). This solution should be made fresh for each use.

Yellow Chromogen Solution (will be on-board mixed)

Note: **Mixed_1, 20A:1B On-Board Mixing** will be used.

Transfer at least 0.9 mL of **Yellow Chromogen** (**SL-035/010**) into a 7 mL **Open Container**, scan, and assign as ***Mix 1, 20:1 Part 1B**.

Transfer 5 mL of **Yellow Chromogen Buffer** (**SL-036/010**) into a 7 mL **Open Container** and assign as ***Mix 1, 20:1 Part 20A**.

Note: **Yellow Chromogen** and **Buffer** can be used again for future staining runs until expiration date provided.

*Hematoxylin

Note: Only one **Hematoxylin** will be used in the protocol.

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

If choosing **Purple Hematoxylin**:

Transfer at least 1950 µL of **Purple Hematoxylin** to appropriately-labeled/registered 6 mL **Titration Container** with insert.

If choosing **Blue Hematoxylin**:

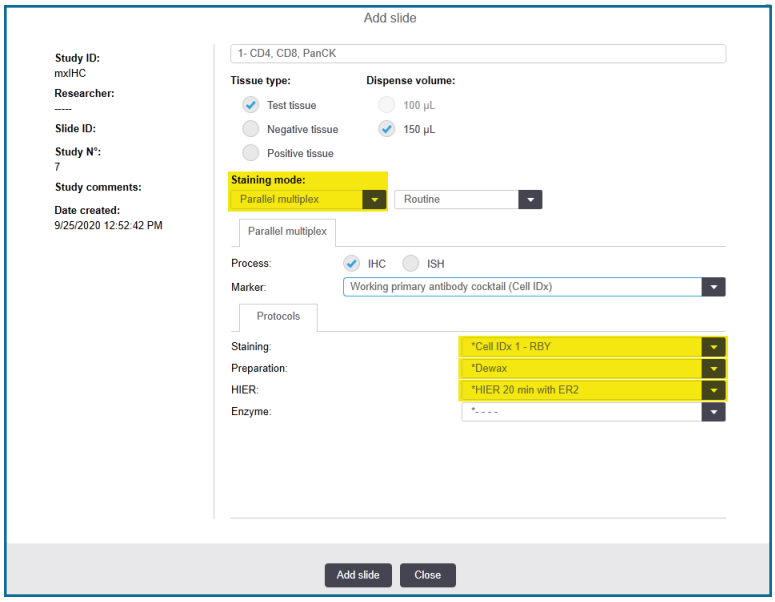
Add 195 µL of **Blue Hematoxylin** into a 6mL **Titration Container** with insert containing 1,755 µL of **DI water**.

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

BOND RX/RX^m Protocols to Use

Slide Set Up

- **Staining Mode**: Parallel multiplex
- **Staining**: *Cell IDx 1- RBY
- **Preparation**: BOND RX *Dewax
- **HIER**: *HIER 20 minutes with ER2



Coverslipping

After BOND RX/RX^m protocol is finished, remove slides from instrument, dip in distilled water.

- Wipe off excess water, mount with aqueous mount, and coverslip
- Slides can also be air dried and mounted with aqueous mounting media or Leica CV Ultra mounting media
- **Avoid alcohol and xylenes**

Customer Support

For assistance or questions, contact **support@cellidx.com** or call us at **(858) 452-5800**.

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A

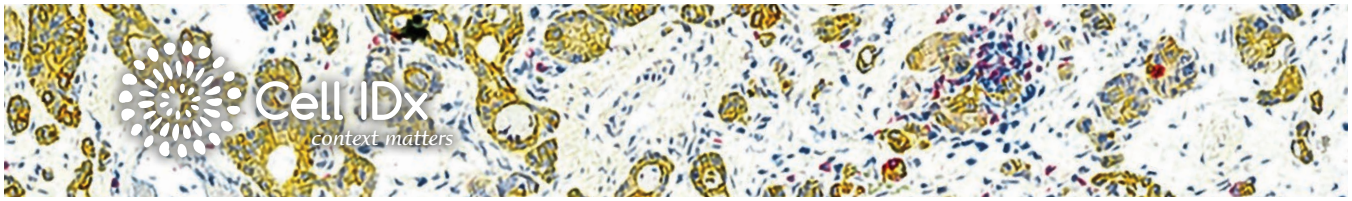
Disclaimer

The products offered here are for research use only. Any commercial application will require a license from Cell IDx, Inc. Cell IDx UltraPlex technology is patented and has multiple patents pending. Please contact Cell IDx for information regarding licensing information. Information in this manual is subject to change without notice and does not constitute a commitment on the part of Cell IDx, Inc. It is supplied on an "as is" basis without any warranty of any kind, either explicit or implied. This documentation is proprietary information and protected by the copyright laws of the United States and international treaties. The manufacturer of this documentation is Cell IDx, Inc.

Safety Information

- WARNING – CHEMICAL HAZARD. Some chemicals used can be potentially hazardous, and can cause injury or illness.
- Read and understand the Material Safety Data Sheets (MSDS) available at www.cellidx.com before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.*, safety glasses, gloves, or clothing). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's clean-up procedures as recommended in the MSDS.
- Comply with all local, state/provincial, and/or national laws and regulations related to chemical storage, handling, and disposal.

For Research Use Only. Not for diagnostic or therapeutic use. © 2019–22 Cell IDx, Inc. Protocol Version 2022.01.27A



Our Vision

Cell IDx is a technology leader in multiplexed tissue profiling, developing highly sensitive and specific chromogenic and fluorescent multiplex immunohistochemistry reagents to meet the needs of precision medicine.

Our UltraPlex platform barcoding technology has enabled the generation of UltraPlex fluorescent and chromogenic multiplex immunohistochemistry panels, providing simultaneous detection of multiple markers in tissue sections and allowing analysis of sub-populations of cells *in situ* in the context of tissue morphology. Multiplex staining is achieved in virtually the same time it takes to perform a single marker stain, enabling truly rapid tissue phenotyping on a large scale.

Our vision is the widespread application of this technology to address both the present and future needs of the research and clinical markets in oncology, immuno-oncology and other disease states. We offer an ever-expanding range of multiplex staining panels as well as rapid development of custom panels, tissue staining, imaging, and analysis services.

We invite you to learn more about our multiplex biomarker technology and see how it can further your tissue profiling and biomarker discovery research. Please contact us to discuss potential collaborations, services, or licensing opportunities.

Cell IDx
context matters®

6197 Cornerstone Court East, Suite 102
San Diego, CA 92121-4718 USA
info@cellidx.com | www.cellidx.com
858.452.5800

For Research Use Only. Not for diagnostic or therapeutic use.
© 2019–22 Cell IDx, Inc. Cell IDx, Inc. UltraPlex, UltraTag, Context Matters, and Cell IDx are trademarks of Cell IDx, Inc. All other trademarks are property of their respective owners. All rights reserved. Printed in the US.
Protocol Version 2022.01.27A