

Customer Notification: Product Configuration and Protocol Change

Catalog Number/s	96-0079 series
Product Name/s	InCELL™ Detection Kit
Effective Date	Jan 20, 2021
Update/s	Kit configuration and sub-component names and part numbers
Affected Document/s	InCELL Detection Kit User Manual (Document No.: 70-380)

Dear Customer,

Further to the advance notification letter issued on July 15, 2020, we have completed the implementation of the sub-component name changes for the InCELL Detection Kit that is included in the eXpress kit. There are no changes in the reagents' formulation and manufacturing process, assay protocols, or ordering process.

To further improve the user experience, we have also simplified the information provided in the "Materials Provided" and "Detection Reagent Volumes per Plate" tables in the InCELL Detection Kit User Manual (Document No.: 70-380). These updates are summarized below.

1. Materials Provided

- The components names have been updated.
- Component volumes provided for the 96-0079 have been increased to better accommodate the use of multi-channel pipettes and reagent reservoirs.
The table now reflects the information as relevant for both InCELL Hunter and InCELL Pulse assays that use the InCELL Detection Kit. The older and updated versions of the Materials Provided table are included below.

Old (Rev 1, page 1)

Catalog Number	96-0079S	96-0079	96-0079L
Number of Plates*	4 Plates	16 Plates	100 Plates
96-well, No. of Data Points	400	1,600	n/a
384-well, No. of Data Points	1,600	6,400	40,000
Kit Components (mL)			
EA Reagent	5	20	200
Lysis Buffer	5	20	200
Substrate Reagent	24	96	800
EA Dilution Buffer**	20	80	800
Total Kit Volume	54	216	2,000

New (Rev 2, page 1)

Catalog Number	96-0079S	96-0079	96-0079L
InCELL Hunter			
Number of Plates per Kit*	2	10	80
Number of Data Points (96-Well/384-Well)	200/800	1,000/4,000	8,000/32,000
InCELL Pulse			
Number of Plates per Kit*	4	20	160
Number of Data Points (96-Well/384-Well)	400/1,600	2000/8,000	12,800/51,200
List of Components			
	Volume per Bottle (mL)		
InCELL EA Reagent	5	25	200
InCELL Lysis Buffer	5	25	200
InCELL Substrate Reagent	24	120	800
InCELL EA Dilution Buffer**	20	100	800

2. Detection Reagent Volumes per plate

- Component volumes required to make the working detection solution have been updated to better accommodate the use of multi-channel pipettes and reagent reservoirs.
- The volumes have been updated to better accommodate the use of multi-channel pipettes and reagent reservoirs.
- Volumes for EA Dilution Buffer have also been included in the updated table.

Old (Rev 1, page 3)

Working Detection Solution Recipes						
Working Detection Solution	EA-1		EA-3		EA-10	
	Volume (µL) per Well or Plate**					
Component	Per Well	Per Plate	Per Well	Per Plate	Per Well	Per Plate
EA Reagent	1	120	3	360	10	1200
Lysis Buffer	10	1200	10	1200	10	1200
Substrate Reagent	49	5880	47	5640	40	4800
Working Detection Solution (Total Volume*)	60	7200	60	7200	60	7200

New (Rev 2, page 5)

Working Detection Solution	EA-1		EA-3		EA-10	
	Volume per Well or Plate (µL)*					
Component	Per Well	Per Plate	Per Well	Per Plate	Per Well	Per Plate
InCELL EA Reagent	1	125	3	375	10	1,250
InCELL EA Dilution Buffer	9	1,125	7	875	0	0
InCELL Lysis Buffer	10	1,250	10	1,250	10	1,250
InCELL Substrate Reagent	40	5,000	40	5,000	40	5,000
Total Volume**	60	7,500	60	7,500	60	7,500

3. Additional Updates

- The document has been updated to reflect Eurofins DiscoverX brand guidelines
- Addition of Table of Contents and Document Revision History

The updated user manual is attached with this letter.



DiscoverX

The Eurofins Discovery PRODUCTS COMPANY

If you have any questions, please contact our technical support team at DRX_SupportUS@EurofinsUS.com.

Sincerely,

Alpana Prasad, Ph.D.
Senior Strategic Portfolio Manager

Attachment: *InCELL Detection Kit User Manual (Document No.: 70-380)*

User Manual

InCELL Detection Kit

For Use with InCell Hunter™ Cell Lines and InCell Pulse™ Starter Kit
Generated with ProLabel® Vectors

Catalog Number: [96-0079 Series](#)

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Overview

The InCELL Detection Kit is to be used with InCELL Hunter™ cell lines (e.g. kinases, epigenetics, and protein binding cell lines) or the InCELL Pulse™ Target Engagement Starter Kit made with ProLabel® expression or cloning vectors. This easy-to-use kit has successfully been run in both 96-well and 384-well microplate format. The resulting chemiluminescent signal can be read with most standard plate readers. To determine instrument compatibility, visit discoverx.com/instrument-compatibility

The InCELL Hunter and Pulse platforms allow the measurement of compound-target engagement based on Eurofins DiscoverX's enzyme fragment complementation (EFC) technology. This technology utilizes β -galactosidase split into two inactive fragments, an enhanced ProLabel (ePL) peptide (or enzyme donor) fragment and an enzyme acceptor (EA) fragment, that complement to form a fully active enzyme. The intracellular target protein in these assays is fused with ePL. Upon addition of a compound that binds the target, protein levels are stabilized or altered within the cell. The detection reagents include a chemiluminescent substrate added with the EA fragment that naturally complements with the ePL tag on the target protein to create an active β -gal enzyme. The resulting active enzyme hydrolyzes the substrate to generate a chemiluminescent signal. The greater the signal corresponds to greater presence of compound-target engagement in the cell.

Materials Provided

Catalog Number	96-0079S	96-0079	96-0079L
InCELL Hunter			
Number of Plates per Kit*	2	10	80
Number of Data Points (96-Well/384-Well)	200/800	1,000/4,000	8,000/32,000
InCELL Pulse			
Number of Plates per Kit*	4	20	160
Number of Data Points (96-Well/384-Well)	400/1,600	2000/8,000	12,800/51,200
List of Components	Volume per Bottle (mL)		
InCELL EA Reagent	5	25	200
InCELL Lysis Buffer	5	25	200
InCELL Substrate Reagent	24	120	800
InCELL EA Dilution Buffer**	20	100	800

*Plates are not included with kits.

**EA Dilution Buffer is only needed for specific InCELL Hunter cell lines. Refer to the cell line-specific datasheet to confirm if the reagent is required for that cell line.

Storage Conditions

Upon receipt, store reagents at -20°C. Thaw reagents at room temperature before use. Thawed reagents are stable for 4 days when stored at 2-8°C. The reagents can tolerate up to three freeze-thaw cycles with no impact on performance.

Additional Materials Required

The following equipment and additional materials are required to perform these assays:

Material	Ordering Information
InCELL Cells	discoverx.com/incell
96-well/384-well Assay Plates	discoverx.com/microplates
Multimode or luminescence plate reader	Refer to the Instrument Compatibility Chart at discoverx.com/instrument-compatibility
Sterile disposable reagent reservoir	ThermoFisher Scientific, Cat. No. 8094 or similar
Single and multichannel micropipettes and pipette tips (10 µL-1000 µL)	
Polypropylene tubes (50 mL and 15 mL)	
Microcentrifuge tubes (1.5 mL)	
Tissue culture disposable pipettes (1 mL-25 mL) and tissue culture flasks (T25 and T75 flasks, etc.)	

Detection Protocol for InCELL Target Engagement Assays

The following assay detection protocols are to be used with InCELL Hunter™ cell lines and the InCELL Pulse™ Target Engagement Starter Kit. If running an assay using an InCELL Hunter cell line (e.g. an InCELL Hunter Kinase Cell Line), refer to the [InCELL Hunter Target Engagement Cell Lines User Manual](#) (Document No. 70-400) and the cell line-specific datasheet for assay preparation details, and follow the procedure outlined in [Detection Reagent Preparation for InCELL Hunter Cell Lines \(384-well format\)](#). When using the InCELL Pulse Target Engagement Starter Kit, refer to the [InCELL Pulse Target Engagement Starter Kit User Manual](#) (Document No. 70-382) for assay development guidelines and protocols, and follow the protocol outlined in [Detection Reagent Preparation for InCELL Pulse Target Engagement Starter Kit](#).

Detection Reagent Preparation for InCELL Hunter Cell Lines (384-well Format)

The following procedure details the assay detection step, in a 384-well format, for InCELL Hunter cell lines. Refer to the [InCELL Hunter Target Engagement Cell Lines User Manual](#) for specific assay preparation details before proceeding.

Detection reagents must be prepared as a working solution prior to use. Certain InCELL Hunter cell lines may require the dilution of EA Reagent with the EA Dilution Buffer. Refer to the cell line-specific datasheet for these requirements.



Refer to the InCELL Hunter cell line-specific datasheet for appropriate EA Reagent dilution requirements before proceeding.

1. If the InCELL Hunter cell line in use does not require dilution of the EA Reagent, then skip this step and proceed to Step 2. If EA dilution is necessary, then dilute the EA reagent by mixing 4 parts of EA Dilution Buffer with 1-part of EA Reagent in a separate tube.
2. Prepare a stock of the working detection solution in a 15 mL polypropylene tube or reagent reservoir by mixing 1-part of EA Reagent (or diluted EA reagent), 1-part of Lysis Buffer, and 4 parts of Substrate Reagent, as listed in the table below.

Working Detection Solution for a 384-Well Format		
Components	Volume Ratio	Volume per Plate (mL)
InCELL EA Reagent*	1	2.5
InCELL Lysis Buffer	1	2.5
InCELL Substrate Reagent	4	10
Total Volume		15

*Dilution of the EA Reagent with EA Dilution Buffer may be required. Refer to the cell line-specific datasheet for requirements.



Do not substitute the InCELL Lysis Buffer with alternate buffers. It is formulated for optimal performance for that assay.



The working detection solution should be used immediately after preparation.

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3. Add 30 μL of the working detection solution to all wells of the assay plate.
4. Incubate the assay plate at room temperature for a standard incubation time of 1 hour in the dark. Some cell lines may require a different incubation period that could be as long as 16 hours (i.e. overnight). Refer to the Functional Performance section on the cell line-specific datasheet. Follow any alternative instructions that may be indicated for the cell line in use.



Working Detection Solution is light sensitive, thus incubation in the dark is necessary.

5. Read samples on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube readers, or 5-10 seconds for imager.

The actual signal characteristics over time are affected by lab conditions such as temperature. The user should establish an optimal read time accordingly. Luminescent readout collects signals from all wavelengths. Some instrument manufacturers may include a cut-off filter at high wavelengths, but usually no wavelength setting is needed for the InCELL Detection kit readout.

Data analysis can be performed using any standard statistical software (e.g. GraphPad Prism, Molecular Devices Softmax Pro, BioTek Instruments Gen5, Microsoft Excel, etc.).

Detection Reagent Preparation for InCELL Pulse Target Engagement Starter Kit

The following procedure details the assay detection step for the InCELL Pulse Target Engagement Starter Kit in a 96-well black PCR plate.

Detection reagents must be prepared as a working solution during the compound incubation step of the assay. Dilution of the EA Reagent may or may not be required during preparation of this working solution. The optimal proportion of EA reagent required in the working solution must be determined during Phase I of the Pulse Assay Development. Refer to the [InCELL Pulse Target Engagement Starter Kit User Manual](#) for instructions on Phase I Assay Development, before proceeding.

Once an optimal formulation of the working detection solution has been established for the assay being developed, it should be used for all subsequent experiments with that particular assay to minimize plate-to-plate, and day-to-day variation.

1. Prepare a stock of the working detection solution in a 15 mL polypropylene tube or reagent reservoir by mixing the EA Reagent, Lysis Buffer, Substrate Reagent, and EA Dilution Buffer (if required). Use the optimal formulation of working detection solution determined during development of the InCELL Pulse assay for the target of interest, as indicated in Phase I Assay Development section in the [InCELL Pulse Target Engagement Starter Kit User Manual](#)

The table below indicates three suggested formulations (i.e. EA-1, EA-3, and EA-10), each differing by the proportion of EA Reagent and EA Dilution Buffer (if required) they contain (e.g. EA-1 contains 1 μL /well of EA Reagent, EA-3 contains 3 μL /well of EA Reagent, etc).

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Working Detection Solution	EA-1		EA-3		EA-10	
	Volume per Well or Plate (µL)*					
Component	Per Well	Per Plate	Per Well	Per Plate	Per Well	Per Plate
InCELL EA Reagent	1	125	3	375	10	1,250
InCELL EA Dilution Buffer	9	1,125	7	875	0	0
InCELL Lysis Buffer	10	1,250	10	1,250	10	1,250
InCELL Substrate Reagent	40	5,000	40	5,000	40	5,000
Total Volume**	60	7,500	60	7,500	60	7,500

*Volumes account for overages and dead volumes.

**The total volume of the EA Reagent and Substrate Reagent (two of the three components included in the working detection solution) should always equal 50 µL (out of the total 60 µL per well volume mix).



Once an optimal working detection solution formulation is determined, use it for all experiments with the Pulse assay developed for the specific target.



Do not substitute the InCELL Lysis Buffer with alternate buffers. It is formulated for optimal performance for that assay.

2. Add 60 µL of working detection solution to all wells of the assay plate.
3. Incubate the assay plate for 1 hour at room temperature in the dark.



The working detection solution is light sensitive, thus incubation in the dark is necessary.

4. Read samples on a standard luminescence plate reader at 0.1 to 1 second per well for photomultiplier tube readers or 5-10 seconds for imager.

The actual signal characteristics over time are affected by lab conditions, such as temperature. The user should establish an optimal read time. Luminescence readout collects signal from all wavelengths. Some instrument manufacturers may include a cut-off filter at longer wavelengths, but usually no wavelength setting is needed for the InCELL Detection Kit readout.

Data analysis can be performed using your choice of statistical analysis software (e.g. GraphPad Prism, Molecular Devices Softmax Pro, Biotek Instruments Gen5, Microsoft Excel, etc.).

For questions on using this product, please contact Technical Support at [1.866.448.4864](tel:1.866.448.4864) or DRX_SupportUS@eurofinsUS.com

Document Revision History

Revision Number	Date Released	Revision Details
0	August 2016	<ul style="list-style-type: none">• New document
1	November 2017	<ul style="list-style-type: none">• Branding updates
2	December 2020	<ul style="list-style-type: none">• Reagent volumes in the detection kit have been updated• Detection reagent volumes per plate have been updated• Branding updates

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