

LavaCell™

Fluorescent Cell Stain

(version A)

Catalog No. 15004

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Introduction

LavaCell™ is a naturally occurring compound from the fungus *Epicoccum nigrum* that can be used to fluorescently stain cells. LavaCell is a small, non-toxic, water-soluble, neutral azaphilone molecule that rapidly diffuses into live or fixed cells and stains cell membranes and lipophilic organelles without staining nucleic acids. Unlike other fluorescent stains, LavaCell does not adversely affect cell growth, is cell permeable, has a long Stokes shift, and unbound dye does not fluoresce¹.

LavaCell has a molecular weight of 410 Daltons and reacts with primary amine groups instantly to produce fluorescent red compounds. LavaCell has a weak green fluorescence in water (520 nm) that shifts to red (610 nm) after reacting with protein. It can be excited at 405, 488 and 532 nm, enabling the use of any common laser^{1, 2}. The staining does not self-quench, and it can be multiplexed with a range of yellow, green and blue-emitting compounds due to LavaCell's characteristic long Stokes shift of at least 100 nm.

LavaCell staining is fast: the plasma membrane is stained in two minutes, and the cytoplasm is stained to saturation in fifteen minutes. The stain has no cytotoxic effect and does not inhibit cellular growth at a concentration of 5 μM over a 24 hour period (Table 1)¹. Because the stain fluoresces much brighter when bound to protein than when unbound, there is little background and washing steps are often not necessary.

product	format	catalog no.
LavaCell™	200 μg	15004

Table 1. *In Vitro* Cytotoxicity Results in Cell Lines at Three Concentrations of LavaCell[®] 3.

Cell Line	Percent Growth at:			Cell Line	Percent Growth at:		
	0.5 μ M	5 μ M	50 μ M		0.5 μ M	5 μ M	50 μ M
<i>Leukemia</i>				<i>Melanoma</i>			
CCRF-CEM	110	95	5	SK-MEL-2B	68	76	41
HL-60 (TB)	85	111	-17	SK-MEL-5	103	104	-6
K-562	100	141	3	UACC-257	94	94	57
MOL T-4	96	99	-26	UACC-62	92	89	-50
RPMI-8226	109	115	3	LOX IMV1	95	100	90
SR	81	102	-8	MALME-3M	100	69	-8
<i>Non-small cell lung cancer</i>				MI4	99	83	-28
A549/ATCC	94	97	10	SK-MEL-2	94	86	37
EKVX	89	86	3	<i>Ovarian cancer</i>			
NCI-H226	86	66	-8	IGROV-1	98	98	12
HOP-62	94	96	1	OVAR-3	68	78	-43
NCI-H3322M	100	84	-15	OVAR-4	104	100	51
NCI-H460	93	90	11	OVAR-5	83	85	-20
NCI-H522	94	91	-22	OVAR-6	91	93	17
<i>Colon cancer</i>				SK-OV-3	72	62	-19
COLO205	82	76	-49	<i>Renal cancer</i>			
HCC-2998	53	68	-28	786-0	95	102	9
HCT-116	92	87	2	ACHN	95	94	87
HCT-15	92	93	73	CAK1-1	97	80	-31
HT29	97	90	48	RXF393	85	86	15
KM12	96	87	-15	SN 12C	87	80	-8
SW-620	103	103	31	TK-10	90	73	20
<i>CNS cancer</i>				UO-31	86	78	20
SF-268	89	83	16	<i>Breast cancer</i>			
SF-295	74	56	-42	MCF-7	95	87	16
SF-539	92	91	4	NCVADR-RES	100	95	-33
SNB-19	65	56	-32	MDA-MB-231/ATCC	76	70	-55
SNB-75	97	89	75	HS 578T	94	86	-15
U251	92	94	85	MDA-MB-435	94	82	-44
<i>Prostate cancer</i>				BT-549	86	78	6
DU-145	91	94	20	T-47D	52	45	-50
PC-3	76	109	35				

Storage Conditions

Reagent	Quantity	Storage / Stability
LavaCell	200 µg (1 mg/ml (2.44 mM) in DMSO)	-20°C for 6 months in the dark

Additional materials required

- Tissue culture supplies
- Formaldehyde (Fixation)
- Fluorescence instrumentation

Protocols – Cell Staining

A. Live Cells

1. Grow cells to desired confluency on suitable coverglass or clear-bottom plate.
2. Prior to staining, wash cells twice with warm (37°C) PBS.
3. Add stain to cells at a final concentration of 2 to 10 µM in culture media for 30 minutes.

Note: If cells are to be maintained in culture after staining, use LavaCell at a concentration of not more than 5 µM. An increase in staining background may develop as LavaCell can stain proteins that are present in serum within serum-containing culture media.

B. Fixed Cells

1. Grow cells to desired confluency and wash twice with cold PBS.
2. Fix cells in chilled PBS containing 4% formaldehyde for 20 minutes at 4°C, then wash twice with cold PBS.
3. Stain cells with LavaCell at a final concentration of 12 to 24 µM in culture medium for 30 minutes in the dark at room temperature.

References

1. Choi, H.-Y. *et al.* (2006) *J. Fluorescence* 16(4): 475-482.
2. Coghlan, D.R. *et al.* (2005) *Org. Lett.* 7(12): 2401-2404.
3. National Cancer Institute Therapeutics Development Program, NCI ID No. D734922/1.

Appendix

Section A. Troubleshooting Guide

Problem/question	Possible Cause	Recommendation
Staining differs across cell population.	Cells may vary in amount of endoplasmic reticulum, Golgi apparatus, etc. which are stained by LavaCell.	.
Background staining present.	Serum-containing cell culture medium.	Wash with serum-free media and stain in serum-free media.

Section B. Related Products

Fluorescent Detection – Protein Quantitation	Units	Catalog No.
ProStain™	1000 rxns	15001

Fluorescent Detection – Albumin Quantitation	Units	Catalog No
Albumin Blue Fluorescent Assay Kit	1 Kit	15002

Fluorescent Detection – Fluorescent Dyes	Units	Catalog No.
Chromeo™ 494 Carboxylic Acid	1 mg	15110
	5 mg	16110
Chromeo™ 494 NHS Ester	1 mg	15111
	5 mg	16111
Chromeo™ 494 Biotin	1 mg	15112
	5 mg	16112
Chromeo™ 494 Streptavidin	1 mg	15113
	5 mg	16113
Chromeo™ 546 Carboxylic Acid	1 mg	15210
	5 mg	16210
Chromeo™ 546 NHS Ester	1 mg	15211
	5 mg	16211
Chromeo™ 546 Biotin	1 mg	15212
	5 mg	16212
Chromeo™ 546 Streptavidin	1 mg	15213
	5 mg	16213
Chromeo™ 642 Carboxylic Acid	1 mg	15310
	5 mg	16310
Chromeo™ 642 NHS Ester	1 mg	15311
	5 mg	16311
Chromeo™ 642 Biotin	1 mg	15312
	5 mg	16312
Chromeo™ 642 Streptavidin	1 mg	15313
	5 mg	16313

Fluorescent Detection – Fluorescent Assays	Units	Catalog No.
ToxCount™	20 x 96 rxns	18010

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the number listed below.

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