

HKCA

Heat-killed preparation of *Candida albicans* - Dectin-1 ligand

Catalog # t1rl-hkca

For research use only

Version # 08J30-MM

PRODUCT INFORMATION

Content:

- 10⁹ freeze-dried cells of heat-killed preparation of *Candida albicans* (HKCA).
- 1 ml sterile endotoxin-free water

Storage:

- HKCA is shipped at room temperature and should be stored at 4°C. Freeze-dried HKCA can be stored at 4°C for at least 1 year.
- Resuspended HKCA can be stored at 4°C for 6 months.

DESCRIPTION

HKCA is a heat-killed preparation of *C.albicans*. HKCA derives from the strain ATCC 10231. HKCA activates the β -glucan specific dectin-1 receptor, which is expressed on phagocytes¹. β -Glucans are glucose polymers found in the cell walls of fungi, such as zymosan (a cell wall preparation of *Saccharomyces cerevisiae*) and *Candida albicans*. Dectin-1 binds and internalizes β -glucans and mediates the production of reactive oxygen species (ROS), activation of NF- κ B and subsequent secretion of proinflammatory cytokines. However, it is now clear that its β -glucan moiety triggers NF- κ B activation only through Dectin-1 as treatment with hot alkali or organic solvents abrogates the TLR2-dependent response^{2,3}. RAW-Blue™ cells express high levels of endogenous Dectin-1 and therefore can be used as a Dectin-1 reporter cell line. Stimulation of RAW-Blue™ cells with depleted zymosan or heat-killed preparations of yeast induces the activation of NF- κ B in a Dectin-dependent manner. NF- κ B activation can be readily monitored as RAW-Blue™ cells stably express an NF- κ B-inducible SEAP reporter gene.

1. Brown GD, et al., 2003. Dectin-1 mediates the biological effects of beta-glucans. J Exp Med. 197: 1119-24. 2. Gantner BN, et al., 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med. 197: 1107-17. 3. Ikeda Y, et al., 2008. Dissociation of Toll-like receptor 2-mediated innate immune response to Zymosan by organic solvent-treatment without loss of Dectin-1 reactivity. Biol Pharm Bull. 31: 13-8. 4. Schindler U, & Baichwal VR., 1994. Three NF- κ B binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol. 14:5820-5831.

METHODS

Preparation of sterile suspension (10⁹ cells/ml)

Stimulation of Dectin-1 can be achieved with HKCA 10⁸ cells/ml.

- Add 1 ml sterile endotoxin-free water (provided) to rehydrate the pellet.

- Vortex 10 sec to homogenize.

Note: Resuspended HKCA results in a milky solution.

HKCA induced NF- κ B activation

- Transfect your cell line with an NF- κ B reporter plasmid, i.e. a plasmid carrying a reporter gene such as SEAP or luciferase, under the control of the NF- κ B-inducible ELAM-1(E-selectin) promoter⁴.

If your cell line does not naturally express dectin-1, cotransfect with a Dectin-1 expressing plasmid, such as pUNO-hdectin1b or pUNO-mdectin1.

Note: Alternatively, evaluate Dectin-1 activation using RAW-Blue™ cells that express the SEAP reporter gene and high levels of endogenous Dectin-1. NF- κ B production in these cells can be easily quantified using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection.

- Twenty-four to forty-eight hours after transfection, stimulate cells with HKCA 10⁸ cells/ml for 24 hours.

- Determine HKCA stimulation on Dectin-1 by assessing reporter gene expression using the appropriate detection system.

RELATED PRODUCTS

Product	Catalog Code
Zymosan	t1rl-zyn
Zymosan depleted	t1rl-dzn
HKSC	t1rl-hksc
pUNO-hDECTIN1a	puno-hdectin1a
pUNO-hDECTIN1b	puno-hdectin1b
pUNO-mDECTIN1	puno-mdectin1
pNiFty2-luc (Zeo)	pnifty2-luc
pNiFty2-SEAP (Zeo)	pnifty2-seap
RAW-Blue™	raw-sp

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