

Validation data for HEK-Blue™ hDectin-1b cells

<http://www.invivogen.com/hek-blue-hdectin1b>

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Version 18C08-NJ

Dectin-1 is alternatively spliced into 2 major isoforms: a full-length A isoform and a 'stalkless' B isoform, which do not induce the same response to soluble and particulate β -glucans. We have engineered HEK-Blue™ cells that stably express high levels of either human Dectin-1a or -1b isoform and an NF- κ B-inducible secreted alkaline phosphatase (SEAP) reporter gene. These cells also express genes involved in the Dectin-1 signaling pathway leading to NF- κ B activation.

HEK-Blue™ hDectin-1b reporter cells are activated by Dectin-1 ligands, however, their responses to soluble and particulate β -glucans differ. While HEK-Blue™ hDectin-1a cells respond well to both particulate and soluble ligands, HEK-Blue™ hDectin-1b cells display a reduced response to particulate ligands and a weak response to soluble ligands. Moreover, HEK-Blue™ hDectin-1b cells do not respond to other CLR ligands such as trehalose-6,6-dibehenate (TDB), a Mincle ligand (Figure 1).

HEK-Blue™ hDectin-1b reporter cells allow to determine the biological activity of soluble and particulate compounds in a specific manner. Of note, soluble ligands such as Laminarin and whole glucan particles (WGP) soluble display an inhibitory activity when cells are incubated with particulate agonists such as Zymosan or Heat Killed *Candida albicans* (HKCA) (Figures 2a and 2b).

Evaluation of NF- κ B responses to Dectin-1 ligands

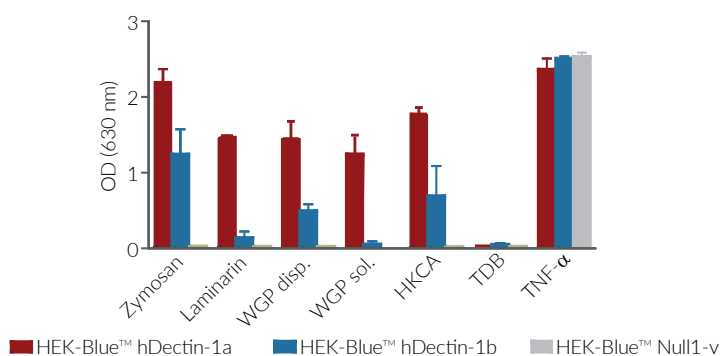


Figure 1: NF- κ B responses of HEK-Blue™ hDectin-1a and -1b and HEK-Blue™ Null 1-v cells (control cell line) to Dectin-1 ligands. Cells were incubated with particulate ligands such as Zymosan (10 μ g/ml), WGP dispersible (100 μ g/ml) and HKCA (3 $\times 10^6$ cells/ml), or soluble ligands such as Laminarin (100 μ g/ml), WGP soluble (10 μ g/ml) or TDB (10 μ g/ml). TNF- α (10 ng/ml) was used as a positive control. After 24h, SEAP activity was assessed in the supernatant using QUANTI-Blue™, by reading the optical density (OD) at 630 nm.

Inhibition of HEK-Blue™ hDectin-1b responses by Laminarin and WGP soluble

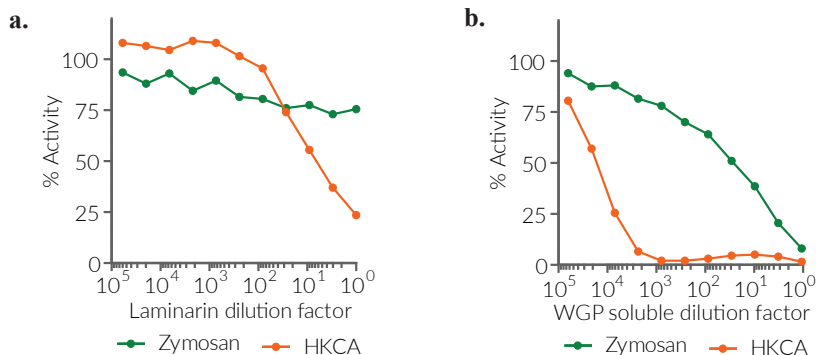


Figure 2: Dose-dependent inhibition HEK-Blue™ hDectin-1b responses by Laminarin and WGP soluble. Cells were incubated with particulate ligands such as Zymosan (30 μ g/ml) or HKCA (3 $\times 10^6$ cells/ml) and soluble ligands such as (a) Laminarin (starting concentration 100 μ g/ml) or (b) WGP soluble (starting concentration 1 mg/ml). After 24h, SEAP activity was assessed in the supernatant using QUANTI-Blue™. Data are presented as the percentage of SEAP activity measured in presence of Zymosan or HKCA without Laminarin or WGP soluble.

TECHNICAL SUPPORT

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