

flashBACGOLD™ Enhanced yields for difficult to express proteins

flashBACGOLD™

Complex secretory or membrane-bound glycoproteins are often more difficult to express using baculovirus and produced in lower amounts compared to cytoplasmic or nuclear proteins^{1,2,3,4,5}.

flashBACGOLD™ is a baculovirus expression vector that has been designed to reduce proteolysis, maximise protein

secretion and improve membrane protein targeting and is based on our patented flashBAC™ system, removing the necessity for plaque-purification.

As such, it is also back-compatible with all existing baculovirus transfer vectors based on homologous recombination in insect cells at the polyhedrin locus.

Technology Overview

Baculovirus genomes contain several auxiliary genes, which are non-essential for replication in insect cell culture. Two of these are chitinase (*chiA*), which encodes an enzyme with exo- and endochitinase activity⁶ and a cathepsin-like cysteine protease (*v-cath*)⁷. In an infected insect, chitinase and cathepsin facilitate host cuticle breakdown and tissue liquefaction at the very late stages of infection, so releasing the virus to infect more hosts⁸.

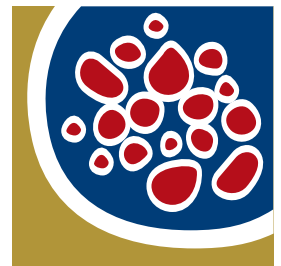
Confocal and electron microscopy observations of insect cells infected with AcMNPV have shown that chitinase is targeted to the endoplasmic reticulum (ER), where it is densely packed in a paracrystalline array, blocking and severely compromising the function and efficacy of the secretory pathway⁹. *V-cath* accumulates in the endoplasmic reticulum at early times post-infection as an inactive proenzyme (pro-*v-cath*) and is then activated by proteolytic cleavage upon cell death, but is sensitive to the cysteine protease inhibitor E-64¹⁰.

It has optimum activity at pH 5.0 - 5.5, although it also shows measurable activity up to pH 7.0¹¹. Chitinase may act as a chaperone for the proper folding of pro-*v-cath* in the ER¹². Together these enzymes compete with the recombinant protein for limiting cellular resources, putting a huge burden on the protein translocational machinery¹³. As a protease, *v-cath* will also degrade susceptible recombinant proteins, particularly in the later stages of infection when the *polh* promoter is most active.

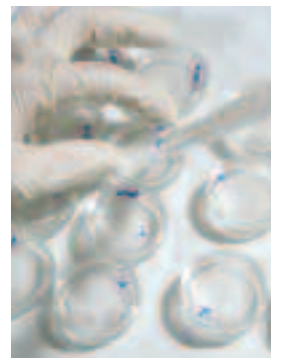
The deletion of both *chiA* and *v-cath* from flashBACGOLD™ has improved the efficacy of the secretory pathway and resulted in a greatly enhanced yield of recombinant proteins that are secreted or membrane targeted (in comparison to recombinant viruses that synthesise *chiA* and *v-cath*). Results also show a significant reduction in degradation of protease-sensitive targets and increased production and stability of some intracellular proteins (manuscript in preparation).

the science of baculovirus expression™

PRODUCTS ARE FOR RESEARCH PURPOSES ONLY, NOT FOR DIAGNOSTIC OR THERAPEUTIC USE

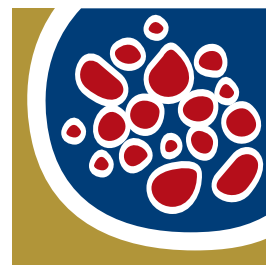


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