**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\(^1\)\(^-\)\(^5\).

**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.

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**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 activity\(^6\) 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 hosts\(^8\).

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 para-crystalline array, blocking and severely compromising the function and efficacy of the secretory pathway\(^9\). 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-64\(^10\).

It has optimum activity at pH 5.0 - 5.5, although it also shows measurable activity up to pH 7.0\(^11\). Chitinase may act as a chaperone for the proper folding of pro-v-cath in the ER\(^12\). Together these enzymes compete with the recombinant protein for limiting cellular resources, putting a huge burden on the protein translocational machinery\(^13\). 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).

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