APOLLO CYTOKINE RESEARCH

Human DNA → Human Cell → Human Proteins hcx

HUMAN PROTEINS
HUMAN SOURCE

THE HUMAN EXPRESS

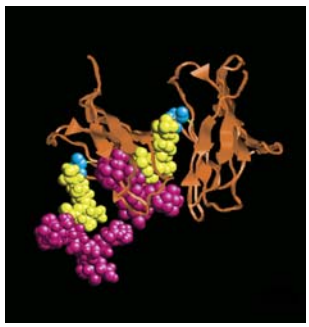
Issue 2 : August 2006

Welcome to your new edition of The Human Express

This issue provides you with more information about our products and their benefits to you. We are also really pleased to share some feedback received from one of our recent customers - the Australian Red Cross Blood Service.

"Using rhIL-2 for human cell proliferation and maintenance, Apollo compared favourably with the Il-2 we are currently using and was more sensitive at lower doses. We were impressed with these results and will be very happy to use Apollo IL-2 in future work."

The Importance of Glycosylation



Apollo Cytokine Research hcx™ proteins are expressed in modified human 293 cells. Since the cells are human, they have the cellular machinery necessary to attach human post-translational modifications (PTMs) to the proteins being expressed.

A major PTM is glycosylation. Glycosylation is the attachment of monosaccharides and oligosaccharides to the protein backbone via a glycosidic linkage.

Our research has shown that in many instances, the presence of human PTMs, such as glycosylation, can have significant impact on your results.

In Search of Superior Human Therapeutic Antibodies

Biologic agents, specifically monoclonal humanized antibodies, are fast becoming the focus of the pharmaceutical industry. They have lower immunogenicity, have longer half-lives, prolonged biologic effects, require less frequent administration and have minimal toxicity.

Ideally the best antigens to use in the immunization step during the development of human therapeutic antibodies would be human proteins expressed from human cells containing human specific glycosylation. As glycosylation patterns are species specific, human protein from other mammalian cells such as NSO or CHO may contain dissimilar glycan patterns. Human proteins produced from *E. coli* do not have any glycosylation.

Therefore, antibodies generated against human proteins derived from other sources apart from human cells may have altered affinities to the target protein and may recognise an epitope that is partially masked by a bulky glycan structure.

Full article >>

Recent Publications : Glycosylation

1) Zucca et al, (2006) Clin Ter 157(1):19-24.

Glycosylated (Lenograstim ,CHO) and non-glycosylated rhG-CSF (Filgrastim, bacterial) were found to differently affect actin polymerization in neutrophils isolated from healthy individuals. Such effects may explain some previous findings concerning both morphology and chemotactic properties and may be due to different effects of the two forms of rhG-CSF on proteins involved in neutrophil motility regulation.

2) Brandner et al, (2006) BBRC 340(3): 836-839

$VEGF_{165}$ binding to endothelial cells is potentiated by glycosaminoglycans (GAGs). The natural ligand heparan sulfate, induced a conformational change only in the glycosylated $VEGF_{165}$ (baculovirus) protein compared to non-glycosylated $VEGF_{165}$ (tunicamycin treated cells). This conformation change may serve to stabilize the structure of the growth factor.

IN THIS ISSUE

USEFUL LINKS

>> Resource Center
>> Why use hcx proteins?
>> FAQs
>> Contact Us

PRODUCTS

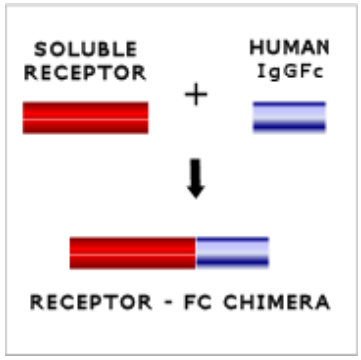
>> View Proteins
>> View ELISA kits

Apollo's hcx™ Proteins

Amphiregulin
BAFF
beta-NGF
BMP-7
CCL2/MCP-1
CCL3/MIP-1 alpha
CCL4/MIP-1 beta
CD209L - Fc Chimera
DCSIGNR - Fc
EPO
EPO R – Fc Chimera
FGF R1 alpha (IIIc) - Fc Chimera
FGF R4 - Fc Chimera
Flt-3 - Fc Chimera
Flt-3 Ligand
G-CSF
GM-CSF
Growth Hormone
Growth Hormone R - Fc Chimera
IFN alpha 2b
IFNAR2 - Fc Chimera
IFN gamma
IGFBP-3
IL-1ra
IL-1 RI - Fc Chimera
IL-2
IL-2 R alpha - Fc Chimera
IL-2 R beta - Fc Chimera
IL-2 R gamma - Fc Chimera

Fc chimeric proteins as potent biological tools for research

Cytokines and particularly the extra cellular domains of cytokine receptors may be expressed as Fc fusion proteins. The Fc domain facilitates the dimerization of the fusion protein via the formation of disulfide bonds. This in turn can increase the binding affinity of particular receptors relative to that of the soluble monomer. This provides the receptor-Fc dimer a number of advantages over the corresponding monomeric receptor equivalents (e.g. soluble Interleukin receptor monomers). Advantages include increased protein half-life, increased ligand binding affinity, and minimising the natural membrane bound receptor complexes.



Full article >>

Protein quantitation of Apollo's hcx™ Proteins

Quantitating the amount of protein present is critical for many applications. At Apollo Cytokine Research we aim to ensure that the amount of protein in our vials is as accurate as possible, thus delivering a product that customers can rely on.

A number of different protein quantitation methods are available, including amino acid analysis (AAA), absorbance at 280 nm, the Bradford and Lowry assays, and enzyme-linked immunosorbent assays (ELISAs). The accuracy of most of these methods relies on using the same pure protein as a standard. Amino acid analysis is an exception, but this method does not work well with heavily glycosylated proteins, measuring absorbance at 280 nm is another.

Find out which method Apollo Cytokine Research uses and why.

Full article >>

Thanks for your help

We recently conducted over 100 telephone interviews to develop our understanding of our market and to gain some valuable insight from our customers. Thank you again to those of you who participated in this research.

These are some of the comments we received about the benefits of hcx™ proteins:

"There's a risk of LPS contamination with E. coli – using a cleaner system would help eliminate these by-products which can kill cells and/or give unspecific results."

"PTM's are very important, including glycosylation. It's critical that generated antibodies bind as closely as possible to both the assay standard protein & the native protein."

"Correct PTM's are essential to obtain valid interactions with ligands and in immunoassays"

"Recombinant bacterial expression is good as a tool, offering good yields but human cell expressed proteins are essential for studying protein-protein interactions to obtain correct/accurate results."

"Human cell expressed would be better for immunization and confirmation of binding avidity to native proteins"

IL-3
IL-3 R alpha - Fc Chimera
IL-4
IL-4 R alpha - Fc Chimera
IL-5
IL-5 R alpha - Fc Chimera
IL-6
IL-7 R alpha - Fc Chimera
IL-8
IL-10
IL-10 R alpha - Fc Chimera
IL-12
L-Selectin - Fc Chimera
Lymphotoxin-alpha
MC-148 - Fc Chimera
MCP-1/CCL2
MIP-1 alpha/CCL3
MIP-1 beta/CCL4
NGF R (209 aa) - Fc Chimera
NGF R (222 aa) - Fc Chimera
Noggin
NT-3
Oncostatin-M
Ox40 - Fc Chimera
SCF
TGF-beta RII - Fc Chimera
TGF-beta 1
Thrombopoietin
TNF-alpha
TNF-beta
TNF RI - Fc Chimera
TNF RII - Fc Chimera
TrkA - Fc Chimera
TrkB - Fc Chimera
TrkC - Fc Chimera
VEGF-C
VEGF-165

Apollo's AccuKine™ ELISA Kits

G-CSF ELISA Kit
GM-CSF ELISA Kit
IL-10 ELISA Kit
IL-2 ELISA Kit
IL-3 ELISA Kit
IL-4 ELISA Kit
IL-6 ELISA Kit
Lymphotoxin-alpha ELISA Kit
TNF-alpha ELISA Kit
TNF-beta ELISA Kit
VEGF-165 ELISA Kit

New Proteins

We are constantly adding new proteins and ELISA kits to our unique range. The following proteins are now available on our website and more will be coming soon. If you are interested in proteins not yet on our product list, please contact us with details about the protein and your requirements.

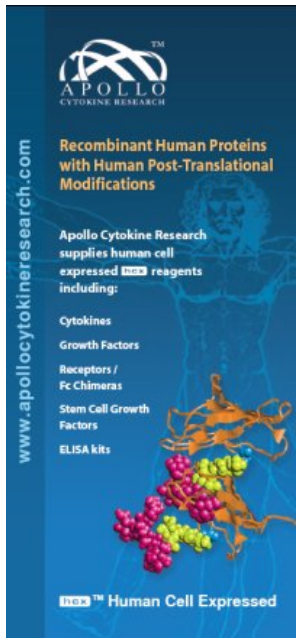
- beta-NGF
- EPO R - Fc Chimera
- IL-12
- NGF R (222 aa) - Fc Chimera
- NT-3
- SCF R – Fc Chimera
- SCF sR
- TGF-beta 1
- Thrombopoietin
- TrkC – Fc Chimera
- VEGF-C

Conference Schedule

We had some really great feedback at the Immunology conference in Boston in May and are looking forward to meeting more of you at the upcoming conferences. Come and have a chat and we can talk to you more about our proteins and their potential for your research.

- **6th International Cytokine Conference 2006**
27-31 August, Vienna, Austria
- **Combio 2006**24-28 September, Brisbane, Queensland, Australia
- **AHMRC 2006**
24-28 September, Melbourne, Victoria, Australia

If you'd like to make an appointment with our team, please email us at contact@apollocytokineresearch.com



For more information on any of the articles introduced in this newsletter, please refer to our website.