

**Recombinant Human Interleukin-4****Quantity:** 50µg**Code:** rHuIL-4-50µg**Batch:** G017/LC1/030526/FP**Exp.date:** 07/2015**Storage:** -20°C**Background**

Interleukin4 (IL4), also known as B cell stimulatory factor1, is a monomeric, approximately 13 kDa 18 kDa Th2 cytokine that shows pleiotropic effects during immune responses (1 – 3). It is a glycosylated polypeptide that contains three intrachain disulfide bridges and adopts a bundled four alpha helix structure (4). Human IL4 is synthesized with a 24 aa signal sequence. Alternate splicing generates an isoform with a 16 aa internal deletion. Human, mouse, and rat IL4 are species specific in their activities (5 7). IL4 exerts its effects through two receptor complexes (8, 9). The type I receptor, which is expressed on hematopoietic cells, is a heterodimer of the ligand binding IL4 Ra and the common gamma chain (a shared subunit of the receptors for IL2, 7, 9, 15, and 21). The type II receptor on nonhematopoietic cells consists of IL4 Ra and IL13 Ra1. The type II receptor also transduces IL13 mediated signals. IL4 is primarily expressed by Th2 biased CD4+ T cells, mast cells, basophils, and eosinophils (1, 2). It promotes cell proliferation, survival, and immunoglobulin class switch to IgG4 and IgE in human B cells, acquisition of the Th2 phenotype by naive CD4+ T cells, priming and chemotaxis of mast cells, eosinophils, and basophils, and the proliferation and activation of epithelial cells (10 13). IL4 plays a dominant role in the development of allergic inflammation and asthma (12, 14).

References:

1. Benczik, M. and S.L. Gaffen (2004) *Immunol. Invest.* 33:109.
2. Chomarat, P. and J. Banchereau (1998) *Int. Rev. Immunol.* 17:1.
3. Yokota, T. et al. (1986) *Proc. Natl. Acad. Sci.* 83:5894.
4. Redfield, C. et al. (1991) *Biochemistry* 30:11029.
5. Ramirez, F. et al. (1988) *J. Immunol. Meth.* 221:141.
6. Leitenberg, D. and T.L. Feldbush (1988) *Cell. Immunol.* 111:451.
7. Mosman, T.R. et al. (1987) *J. Immunol.* 138:1813.
8. Mueller, T.D. et al. (2002) *Biochim. Biophys. Acta* 1592:237.
9. Nelms, K. et al. (1999) *Annu. Rev. Immunol.* 17:701.
10. Paludan, S.R. (1998) *Scand. J. Immunol.* 48:459.
11. Corthay, A. (2006) *Scand. J. Immunol.* 64:93.
12. Ryan, J.J. et al. (2007) *Crit. Rev. Immunol.* 27:15.
13. Grone, A. (2002) *Vet. Immunol. Immunopathol.* 88:1.
14. Rosenberg, H.F. et al. (2007) *J. Allergy Clin. Immunol.* 119:1303.

Source :	E.coli
Appearance :	Colorless, clear liquid free of particles
Identity :	1 band at 15kDa as measured by SDS-PAGE/Western Blot
Specific activity :	12.4 x 10 exp6 units / mg compared to NIBSC standard (Bioassay) 12.4 10**6U/mg (NIBSC standard avec CT.h4S cells)
Endotoxin content:	< 0.1EU/µg (LAL)
Protein content:	50±10 µg/Vial (Lowry/µBCA)
Trehalose:	6±0.5 mg/ml (HPLC)
Sterility test:	Absence of growth (FTM (30-35°C)) Absence of growth (TSB (20-25°C))
Abnormal toxicity: General Safety	No weight loss, no abnormal reaction in mice No weight loss, no abnormal reaction in guinea pigs
Physical state :	Freeze-dried
Reconstruction :	Use 500µL water for injection in class A environment in order to keep the GMP Grade
Stability :	12 months at -20°C to -80°C At least 3 months after reconstruction when stored at -20°C to -80°C
Packaging unit :	50 µg protein (Lowry test)
Purity :	>98% as determined by SDS-PAGE and HPLC

GMP

GENTAUR rh IL-4 is manufactured in full compliance with cGMP in facilities approved by the Belgian Ministry of Health for the production and storage of medicinal products. The manufacturing process does not involve the use of products of animal origin.

Use

GENTAUR rh IL-4 is not an approved medicinal product and cannot be injected as such to patients.

Supplementary data:

STABILITY TESTING

NB: Time 0 = Final Container

After 7 days at 37°C

TEST	APPLICATION	SPECIFICATION	RESULT	CONCL.
Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 16 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 16 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	10,9 10 ⁶ U/mg	PASS
Abnormal toxicity / General safety	on mice	No weight loss, no abnormal Reaction	Absence of symptoms	PASS
Abnormal toxicity / General safety	on guinea-pigs	No weight loss, no abnormal Reaction	Absence of symptoms	PASS
Sterility test	FTM 30-35°C	Absence of growth	Absence of growth	PASS
Sterility test	TSB 20-25°C	Absence of growth	Absence of growth	PASS

After 3 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 16 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 16 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	11,8 10 ⁶ U/mg	PASS

After 6 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 15 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	20,7 10 ⁶ U/mg	PASS

After 9 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 15 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	12,0 10 ⁶ U/mg	PASS

After 12 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 15 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	13,8 10 ⁶ U/mg	PASS

After 18 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 15 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	13,2 10 ⁶ U/mg	PASS

After 24 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band between 12 and 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	2 band between 12 and 15 kDa	PASS
Activity	Bioassay	> 5 10 ⁶ U/mg	12,5 10 ⁶ U/mg	PASS
Abnormal toxicity / General safety	on mice	No weight loss, no abnormal Reaction	Absence	PASS
Abnormal toxicity / General safety	on guinea-pigs	No weight loss, no abnormal Reaction	Absence	PASS
Sterility test	FTM 30-35°C	Absence of growth	Absence of growth	PASS
Sterility test	TSB 20-25°C	Absence of growth	Absence of growth	PASS

After 36 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band at about 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	1band at about 15 kDa	PASS

After 48 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band at about 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	1band at about 15 kDa	PASS

After 60 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band at about 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	1band at about 15 kDa	PASS

After 72 months at -20°C

Electrophoretical pattern	SDS-PAGE	1band between 12 and 16 kDa	1band at about 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	1band at about 15 kDa	PASS

ACTIVITY TESTING:

The specific activity has been determined 9 times in total:

7 determinations were done with CT.h4S cells 2 with TF1 cells

One determination has been excluded: 20.7 10**6U/mg

6 determination give average 12.4 10**6 U/mg

2 determinations in new conditions gave the average of 6.6 10**6U/mg

Depending on the variable conditions cells and of the supernatant used, cytokine content, we obtain different values of activity that all confirm that the activity of this highly pure GMP IL-4 is over 5 10**6 U/mg

lot G017/LC1/030526

04-GMPHuIL4-50 µg
GMP-IL-4, 50 µg

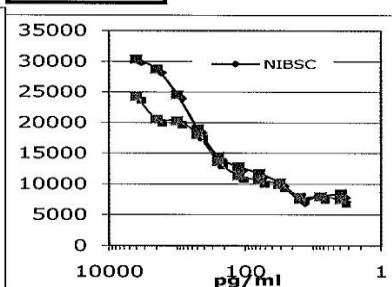
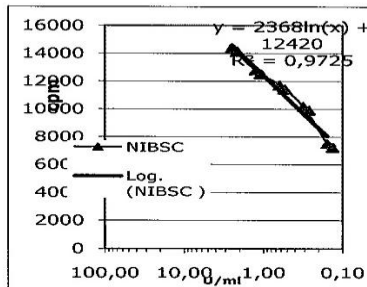


pg/ml	cpm	y=a ln(x)+b		m(x)=(y-b)/a	U/cup	U/mg
4000,0	24270	a	2368	5,00	149,0	37
2000,0	20545	b	12420	3,43	30,9	15
1000,0	20283			3,32	27,7	28
500,0	18018			2,36	10,6	21
250,0	13590			0,49	1,6	6,6
125,0	11325			-0,46	0,6	5,0
62,5	10585			-0,77	0,5	7,4
31,3	9904			-1,06	0,3	11
15,6	7844			-1,93	0,1	9
7,8	7935			-1,89	0,2	19
3,9	7413			-2,11	0,1	31

NIBSC standard TF1 cells

Average **6,3**
S.D. **1,2**

6,3 10**6U/mg



Brussels 1090, 8 september 2010
Lieven GEVAERT, bio-engineer



04-GMPHuIL4-50 µg
GMP-IL-4, 50 µg

lot G017/LC1/030526

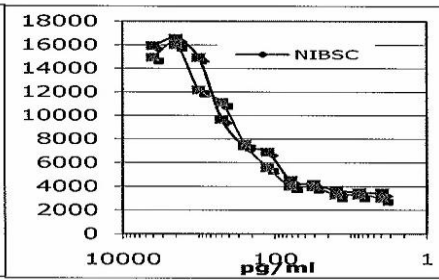
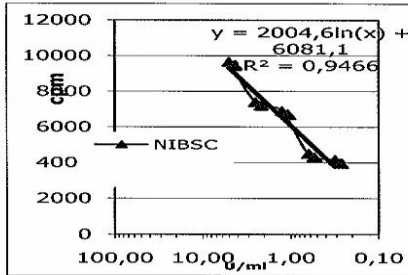


pg/ml	cpm	y=aln(x)+b		ln(x)=(y-b)/a	U/cup	U/ng
4000,0	14946	a	2004	-4,42	83,4	21
2000,0	16026	b	6081	-4,86	143,0	71
1000,0	12174			3,04	20,9	21
500,0	11121			2,51	12,4	25
250,0	7564			0,74	2,1	8,4
125,0	5584			-0,25	0,8	6,2
62,5	4018			-1,03	0,4	5,7
31,3	3972			-1,05	0,3	11
15,6	3278			-1,40	0,2	16
7,8	3293			-1,39	0,2	32
3,9	3003			-1,54	0,2	55

NIBSC standard TF1 cells

Average: **6,8**
S.D.: **1,4**

6,8 10**6 U/mg



Brussels 1090, 20 august 2010
Lieven GEVAERT, bio-engineer



GMP

GENTAUR rh GMP IL-4 is manufactured in full compliance with cGMP in facilities approved by the Belgian Ministry of Health for the production and storage of medicinal products. GMP production at HENOGEN SA and GMP Lyophilisation at GSK Inc, Rixensart.



REÇU LE 08 OCT. 2002



AUTORISATION N° : 1.535

Accordée le:

07 OCT. 2002

En application de l'article 2 de l'arrêté royal du 6 juin 1960 concernant la fabrication, la distribution en gros et la dispensation des médicaments.

La société: HENOGEN
Siège Social: Rue des Prof. Jeener et Brachet 12 – 6041 GOSELIES

Représentée par: M. BOLLEN, Directeur-Général
Est autorisé(e) à:

• **fabriquer:**

- les médicaments non présentés sous forme de spécialités indiquées

sur l'annexe D (comprenant 1 feuille)

Sur chaque annexe est indiqué l'endroit où ont lieu des opérations renseignées ci-dessus. Toute modification que la personne autorisée désireait voir apporter aux dénominations, lieux ou autres renseignements figurant sur la présente autorisation (annexes comprises) rend nécessaire le renouvellement de celle-ci.

POUR LE MINISTRE DE LA SANTE PUBLIQUE,
LE CONSEILLER GENERAL,

REDEVANCE DUE :
MONTANT : 1242
POUR : autorisation n° 1535

Use

GENTAUR rh GMP IL-4 is not an approved medicinal product and cannot be injected as such to patients. However this GMP GM-CSF is used in clinical tests for DC Therapy today in Belgium, France, Denmark, USA and Japan.
CE CERTIFIED FOR EX VIVO CELL CULTURE AND DC THERAPY CLINICAL TESTS