

GMP Recombinant Human Interleukin - 4 (rHuIL - 4)

Quantity : 50µg

Code : GMP rHuIL-4-50µg

Batch : G017/LC1/030526/PV

Storage: -20°C

Price: 483 Euro

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.

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.

Source	E.coli
Appearance	Colourless, 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)
Endotoxin content	< 0.1EU/µg (LAL)
Protein content	50 ± 10 µg/vial (Lowry/µBCA)
Trehalose	6±0.5 mg/ml (HPLC)
Sterility	Absence of growth (FTM (30° - 35°C)) Absence of growth (TSB (20° - 25°C))
Abnormal toxicity	No weight loss, no abnormal reaction in mice
General Growth	No weight loss, no abnormal reaction in guinea pigs
Physical state	Freeze-dried
Stability	12 months at -20°C to -80°C At least 3 months after reconstruction when stored at -20°C to - 80°C
Reconstruction	Use 500µL water of injection
Packaging unit	50 µg protein (Lowry test)

STABILITY TESTING



After 7 days at 37°C

TEST	APPLICATION	SPECIFICATION	RESULT	CONCL.
Electrophoretical pattern	SDS-PAGE	1 band between 12 and 16 kDa	1 band 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	on mice	No weight loss, no abnormal reaction	Absence of symptoms	PASS
	on guinea-pigs	No weight loss, no abnormal reaction	Absence of symptoms	PASS
Sterile test	FTM 30° - 35°C	Absence of growth	Absence of growth	PASS
	TSB 20° - 25°C	Absence of growth	Absence of growth	PASS

After 3 months at -20°C

Electrophoretical pattern	SDS-PAGE	1 band between 12 and 16 kDa	1 band 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	1 band between 12 and 16 kDa	1 band 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

TEST	APPLICATION	SPECIFICATION	RESULT	CONCL.
After 9 months at -20°C				
Electrophoretical pattern	SDS-PAGE	1 band between 12 and 16 kDa	1 band 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	1 band between 12 and 16 kDa	1 band 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	1 band between 12 and 16 kDa	1 band 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	1 band between 12 and 16 kDa	1 band 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	on mice on guinea-pigs	No weight loss, no abnormal reaction	Absence	PASS
Sterile test	FTM 30° - 35°C TSB 20° - 25°C	Absence of growth	Absence of growth	PASS
After 36 months at -20°C				
Electrophoretical pattern	SDS-PAGE	1 band between 12 and 16 kDa	1 band 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	on mice on guinea-pigs	No weight loss, no abnormal reaction	Absence	PASS
Sterile test	FTM 30° - 35°C TSB 20° - 25°C	Absence of growth	Absence of growth	PASS
After 48 months at -20°C				
Electrophoretical pattern	SDS-PAGE	1 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Activity	Bioassay	1 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Electrophoretical pattern	SDS-PAGE	2 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Identity	Western Blot	1 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Activity	Bioassay	2 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Electrophoretical pattern	SDS-PAGE	1 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Identity	Western Blot	2 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Activity	Bioassay	1 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Electrophoretical pattern	SDS-PAGE	2 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Identity	Western Blot	1 band between 12 and 16 kDa	1 band at about 15 kDa	PASS
Activity	Bioassay	2 band between 12 and 16 kDa	1 band at about 15 kDa	PASS

ACTIVITY TESTING:

The specific activity has been determined 9 times in total:

- 7 determinations where done with CT.H4S cells 2 with TF1 cells
- One determination has been excluded; 20.7 10⁶ U/mg
- 6 determination give average 12.4 10⁶ U/mg
- 2 determinations in new conditions gave the average of 6.6 10⁶ U/mg

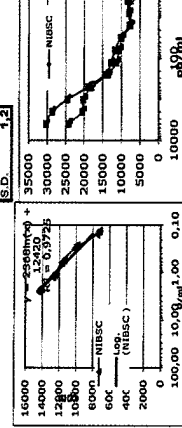
Depending on the variable conditions cells and of the supernatant used, cytokine content, we obtain different values of activity that all confirm that the activity of this highly pure GMP IL-4 is over 5 10⁶ U/mg

lot 0017ALC1030528

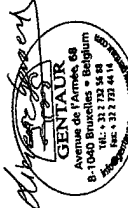
04-GMP/IL-4-30 mg
GMP-IL-4, 30 µg

LOT	GM	VE	IL-4	CONC	U/MG	U/ML
4000.0	24370	2380	3101	30.0	30.0	30.0
2000.0	20545	3.43	30.0	15	15	15
1000.0	20283	3.42	27.7	20	20	20
200.0	13360	0.48	1.8	6.0	6.0	6.0
128.0	11326	-0.48	0.6	5.0	5.0	5.0
62.8	10893	-0.77	0.5	7.4	7.4	7.4
15.8	7844	-1.05	0.3	10	10	10
7.8	7936	-1.89	0.2	19	19	19
3.8	7413	-2.11	0.1	31	31	31

NIBSC standard TP1 cells



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

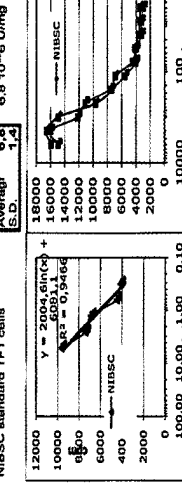


lot 0017ALC1030528

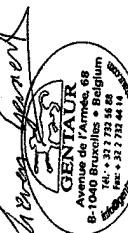
04-GMP/IL-4-30 mg
GMP-IL-4, 30 µg

LOT	GM	VE	IL-4	CONC	U/MG	U/ML
4000.0	18480	4.92	49.2	49.2	49.2	49.2
2000.0	18026	4.56	45.6	45.6	45.6	45.6
1000.0	12174	2.04	20.4	20.4	20.4	20.4
200.0	7854	0.74	7.4	7.4	7.4	7.4
128.0	6584	-0.26	0.8	6.2	6.2	6.2
62.8	5072	-1.03	0.3	9.7	9.7	9.7
15.8	3072	-1.40	0.2	18	18	18
7.8	3263	-1.39	0.2	32	32	32
3.8	3263	-1.94	0.2	58	58	58

NIBSC standard TP1 cells



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.

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.
DC CERTIFIED FOR EX VIVO CELL CULTURE AND DC THERAPY CLINICAL TESTS



Ministère des Affaires Sociales,
de la Santé Publique et de
l'Environnement

RECU LE 06 OCT. 2002
Autorisée le: 07 OCT. 2002

INSPECTION GENERALE
DE LA PHARMACIE

La société: HENOGEN
Siège Social: Rue des Prof. Jansen et Fresher 12 - 6041 GROSSELLES

Représenté par: M BOLLLEN, Directeur-Général
Est autorisé(e) à: fabriquer

les médicaments non présentés sous forme de spécialités indiquées sur l'annexe D (composant I feuille)

Sur chaque étiquette est indiqué l'endroit où ont lieu des opérations réglementaires ci-dessus. Toute modification de ces données doit être autorisée par le Ministère des Affaires Sociales, de la Santé Publique et de l'Environnement (faute sur la présente autorisation (annexes comprises) rend nécessaire le renouvellement de celle-ci)

POUR LE MINISTRE DE LA SANTE PUBLIQUE,
LE CONSULTEUR GENERAL,
[Signature]

REDEVANCE DUE: _____
MONTANT: _____
POUR: _____