

the glass filter is poured an eluent (50 ml) consisting of 0.1 M phosphate buffer (pH 7.5) containing 1.0 M sodium chloride and 3.0 M sodium thiocyanate. After maintaining for 5 minutes, the gel is sucked to elute LPF-HA. The eluted LPF-HA is dialyzed against 0.01 M phosphate buffer (pH 7.5) to remove sodium thiocyanate. The LPF-HA thus obtained is analyzed in the same manner as in Example 1.

The LPF-HA is subjected to Limurus test to measure the endotoxin of *B. pertussis* and further to the measurement of leucocytosis-promoting activity in mice and other physiological activities. These results are shown in Table 2 and Table 3.

As is clear from the results, in case of using a gel of the denatured ceruloplasmin (as a ligand) of the present invention, a highly pure LPF-HA is obtained in a high yield and the endotoxin of *B. pertussis* is almost removed (see Limurus test). On the contrary, in case of the reference gel of haptoglobin (as a ligand), the endotoxin is insufficiently removed. The LPF-HAs thus obtained are subjected to pyrogen test in rabbits. As a result, the LPF-HAs purified by the gels of the denatured ceruloplasmins (as a ligand) of the present invention are all negative, which means that the endotoxin is sufficiently removed.

TABLE 2

Kind of ligand for affinity gel	Amount of eluent (ml)	LPF—HA activity × 10 ⁴ unit/ml	HA value	Specific activity	Purity (%)	Yield (%)	Limurus test (W)
NaCN—denatured ceruloplasmin	50	11.1	1024	157.4	92.5	99.1	100
1-Ascorbic acid-denatured ceruloplasmin	50	11.7	1024	169.6	96.8	104.5	100
Heat treated ceruloplasmin	50	11.4	1024	155.5	92.6	101.8	100
Haptoglobin	50	7.8	512	125.0	80.5	70.3	100

[Note]:

The starting supernatant of a culture medium has an endotoxin content of 1.0×10^5 W by Limurus test.

TABLE 3

Kind of ligand for affinity gel	Leucocytosis-promoting activity in mice (*1) (LPU/ml)	Histamine-sensitizing activity in mice (*2) (HSU/ml)	HSD ₅₀ (g)	Pyrogen test in rabbit (°C.)
Starting material (Supernatant of culture medium of <i>B. pertussis</i>)	15.0	3.0	—	3.5
LPF—HA purified by heat-treated ceruloplasmin gel	1238.4	385.6	0.02	0.1
LPF—HA purified by haptoglobin	675.3	225.4	0.04	1.0

(*1) and (*2): Minimum Requirement of Biological Products, Ministry of Health and Welfare, Japan, #287, 1981.

What is claimed is:

1. In a method for production of pure LPF-HA (Leucocytosis-promoting factor hemagglutinin) comprising subjecting a culture medium of *Bordetella pertussis* to an affinity chromatography and eluting LPF-HA adsorbed on the affinity chromatography gel with an eluent, the improvement comprises subjecting the culture medium to an affinity chromatography using a

heat-treated ceruloplasmin as a ligand for the affinity chromatography gel.

2. The method according to claim 1, wherein the affinity chromatography is carried out on a culture medium having pH 4.0 to 10.0.

3. The method according to claim 1, wherein the heat-treated ceruloplasmin is obtained by heat treatment of a human- or other animal-origin ceruloplasmin at 60° to 85° C. for 1 to 24 hours.

4. The method according to claim 1, wherein the heat-treated ceruloplasmin is obtained by heat-treating a human- or other animal-origin ceruloplasmin and further treating it with a denaturing agent selected from the group consisting of a sulfide, a reducing sugar, a cyano compound, and a chelating agent, whereby copper ion is reduced or a part or all of copper ion is removed.

5. The method according to claim 4, wherein the denaturing agent is a member selected from the group consisting of sodium sulfide, ammonium sulfide, 1-ascorbic acid, D-glucose, D-fructose, maltose, lactose, acetaldehyde, formic acid, oxalic acid, mercaptoethanol, diethyldithiocarbamate, sodium cyanide, sodium thiocyanate, EDTA, nitrotriacetic acid, and triethylenetetraminehexaacetic acid.

6. The method according to claim 5, wherein the denaturing agent is a member selected from the group consisting of sodium cyanide and 1-ascorbic acid.

7. The method according to claim 1, wherein the affinity chromatography is carried out by a column method comprising passing a culture medium of *B. pertussis* through a column packed with an affinity chromatography gel using a heat-treated ceruloplasmin as a ligand at a flow rate of 10 to 500 ml/cm²/hour, washing the column with a buffer having a pH 4.0 to 9.0 and a specific electric conductivity of 10 to 150 ms/cm, followed by eluting the adsorbed LPF-HA with an eluent selected from the group consisting of chaotropic base, an ethylene glycol, dioxane, urea, guanidine hydrochloride, and EDTA.

8. The method according to claim 1, wherein the affinity chromatography is carried out by a batch method comprising stirring a mixture of a culture medium of *B. pertussis* and an affinity chromatography gel using a heat-treated ceruloplasmin as a ligand for 30 minutes to 3 hours, washing the mixture with a buffer having a pH 4.0 to 9.0 and a specific electric conductivity of 10 to 150 ms/cm, followed by eluting the adsorbed LPF-HA with an eluent selected from the group consisting of chaotropic base, an ethylene glycol, dioxane, urea, guanidine hydrochloride, and EDTA.

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