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14. その他必要な事項

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(2) 表

表 1. 遺伝子製剤 IAB-1 の規格

試験項目	規格
性状	白色の塊ないし粉末(凍結乾燥剤)
確認試験	257～261nm に極大吸収
pH	7.0～7.6
浸透圧比	1.0～1.4(凍結乾燥剤)
純度試験(類縁物質)	15%以下
発熱性物質試験	陰性
無菌試験	適合
生物活性(pDRSV-IFN β 15ng 当たり)	
ヒトβ型インターフェロン産生量	150 国際単位/ml 以上
細胞増殖抑制率	30%以上
定量	
pDRSV-IFN β	0.10～0.17mg/ml(凍結乾燥剤)
リポソーム膜成分	
TMAG	3.6～7.2mg/mg-DNA
DLPC	7.8～13.8mg/mg-DNA
DOPE	9.0～16.8mg/mg-DNA
大腸菌染色体 DNA	10 μ g/mg プラスミド DNA 以下
タンパク質	10 μ g/mg プラスミド DNA 以下
エンドトキシン	10EU/mg プラスミド DNA 以下

表2-1　ＩＡＢ－１凍結乾燥製剤の規格及び試験方法

（１）性状

本品は白色の塊又は粉末である．

（２）確認試験

本品 1 個をとり，内容物に水 1 mL を加えて懸濁する．この液をメタノール溶液で希釈（1→10）した液につき，220 nm ～ 320 nm の吸収スペクトルを測定するとき，波長 257～ 261 nm に吸収の極大を認める．

（３）ｐＨ

本品 1 個をとり，内容物に水 1 mL を加えて懸濁した液のｐＨは 7.0～7.6 である．

（４）浸透圧比

本品 1 個をとり，内容物に水 1 mL を加えて懸濁した液の浸透圧比は 1.0 ～ 1.4 である．

（５）純度試験（プラスミドＤＮＡ分解物）

本品 1 個をとり，内容物に水 1 mL を加えて懸濁した液 20 μL に可溶化緩衝液 10 μL を加えて溶かし，試料溶液とする．この液 15 μL を正確にとり，50 w/v% グリセリン水溶液 20 μL，水 35 μL 及び可溶化緩衝液 30 μL を加えて混合し，標準溶液とする．試料溶液及び標準溶液 12 μL につき，アガロースゲルを用いて以下の条件で電気泳動を行う．泳動後，トランスイルミネーター上でデンシトグラムを測定するとき，試料溶液の主バンド以外のバンドのピークの合計面積値は標準溶液から得たピーク面積値より大きくない．

泳動条件

装置：コスモ・バイオ製ミューピッド２サブマリン型電気泳動装置

電圧：50 V

泳動時間：70 分

ゲル：0.75 w/v% アガロースゲル

泳動緩衝液：0.5 μg/mL 臭化エチジウム混合 TAE 緩衝液

表2-2 ＩＡＢ－１凍結乾燥製剤の規格及び試験方法

（６）発熱性物質試験

本品 1 個をとり，内容物に水 1 mL を加えて懸濁する．この液に生理食塩液を加え，1 mL 中に pDRSV-IFNβ 60 μg を含むように調製した液 1 mL/kg を投与し，発熱性物質試験を行うとき，これに適合する．

（７）無菌試験

本品 10 個 をとり，1 w/v % デオキシコール酸ナトリウム水溶液 3 mL をそれぞれ加えて内容物を溶解した液につき，無菌試験法のメンブランフィルター法により試験を行うとき，これに適合する．

（８）生物活性試験

１）ヒトインターフェロンβ産生試験

本品 1 個をとり，内容物に水 1 mL を加えて懸濁する．次いで，プラスチック製滅菌培養プレートに接種し(3×10^3 cells/100 μL/ウエル), 37 ℃ で一晩培養した U251 SP 細胞の上清を静かに除き，本品の表示量に従い調製した pDRSV-IFNβ 15 ng に対応する量を含むダルベッコ MEM 培地 0.1 mL を加え，37 ℃ で 48 時間培養後,上清をとり，ELISA 法により培養上清中のヒトインターフェロンβ量を求めるとき，150 国際単位/mL 以上である．

２）細胞増殖抑制試験

本品 1 個をとり，内容物に水 1 mL を加えて懸濁する．次いで，プラスチック製滅菌培養プレートに接種し(3×10^3 cells/100 μL/ウエル), 37 ℃ で一晩培養した U251 SP 細胞の上清を静かに除き，本品の表示量に従い調製した pDRSV-IFNβ 15 ng に対応する量を含むダルベッコ MEM 培地 0.1 mL を加え，37 ℃ で 48 時間培養後．上清を除去する.PBS を用いて細胞及び ウエル を 2 回洗浄後，0.5 w/v% クリスタルバイオレット・20 vol% メタノール溶液 0.1 mL を加え，室温で 15 分間染色する．次いで，細胞及びウエルを水で余分な色素を洗浄し，乾燥させた後，33 vol% 酢酸 0.1 mL を用いて細胞からクリスタルバイオレットを抽出する．この液につきマイクロプレートリーダーを用い 600 nm の吸光度を測定し，細胞増殖抑制率を求めるとき，30 % 以上である．

表2-3 ＩＡＢ－１凍結乾燥製剤の規格及び試験方法

（9）定量

1）pDRSV-IFNβ

本品 1 個をとり，内容物に水 1 mL を加えて懸濁する．この液 0.5 mL を正確にとり，メタノールを加えて正確に 5 mL とし，試料溶液とする．別に pDRSV-IFNβ標準原液 0.1 mL を正確にとり，膜成分定量用標準液原液 0.6 mL 及び 30 w/v% 白糖溶液 0.15 mL をそれぞれ正確に加え、メタノールを加えて正確に 5 mL とし，標準溶液とする．試料溶液及び標準溶液につき，波長 259 nm における吸光度 A_T及び A_Sをそれぞれ測定し，以下の式に挿入して本品 1 個に含まれる pDRSV-IFNβの量を求めるとき，0.10 ～ 0.17 mg の pDRSV-IFNβを含む．

pDRSV-IFNβの量（mg）＝ $0.02 \times A_T / A_S \times 10$

2）リポソーム膜成分（TMAG，DLPC，DOPE）

本品 1 個をとり，内容物に水 1 mL を加えて懸濁する．この液 0.5 mL を正確にとり、メタノール 4 mL を加えて溶かした後，直ちに 20 mmol/L リン酸ナトリウム緩衝液 (pH 2.6) 0.25 mL を加え，更にメタノールを加えて正確に 5 mL とし，試料溶液とする．別に TMAG 31 mg，DLPC 62 mg 及び DOPE 74 mg を精密に量り，メタノールを加えて溶解し正確に 50 mL とする．次いで，この液 1 mL を正確に量り，メタノールをそれぞれ加え，正確に 5 mL，10 mL，20 mL とし，検量線用標準溶液とする．試料溶液及び検量線用標準溶液 40 μL につき，次の条件で液体クロマトグラフ法により試験を行う．各検量線標準溶液から得られたそれぞれの膜成分のピーク面積値より検量線を作成し，この検量線により試料溶液から得られた各膜成分量を求めるとき，pDRSV-IFNβ 1 mg 当たり，TMAG，DLPC，DOPE をそれぞれ 3.6 ～ 7.2 mg，7.8 ～ 13.8 mg，9.0 ～ 16.8 mg を含む．

操作条件

検出器：紫外吸光光度計（測定波長：210 nm）

カラム：内径約 4 mm，長さ約 30 cm のステンレス管に 7 μm の液体クロマトグラフ用シリカゲルを充填する．(Wakosil-7SIL-120)

カラム温度：40 ℃ 付近の一定温度

移動相：アセトニトリル／3 mmol/L 過塩素酸ナトリウム・10 mmol/L リン酸ナトリウム緩衝液(pH 2.6)混液（171:29）

流量：DOPE，TMAG，DLPC の保持時間がそれぞれ約 6.5，9，20 分になるように調製する．

表2-4 ＩＡＢ－１凍結乾燥製剤の規格及び試験方法

(10) 試液及び調製法

可溶化緩衝液：Triton X-100 10 g に 0.5 mol/L EDTA 溶液（pH 8.0）20 mL 及び水を加えて 100 mL とする.

TAE 緩衝液：トリス 242 g に 酢酸 57.1 mL, 0.5 mol/L EDTA 溶液 100 mL 及び水を加えて 1000 mL とする. この液を用時 50 倍希釈して泳動に用いる.

20 mmol/L リン酸塩緩衝液(pH 2.6)：無水リン酸一ナトリウム 1.2 g を 400mL の水に溶解し, 1 mol/L リン酸を加えて pH を 2.6 にした後, 水で 500 mL にする.

移動相：20 mmol/L リン酸ナトリウム緩衝液（pH 2.6）100 mL に過塩素酸ナトリウム 73.5 mg を加えて溶かした後, 水で 200 mL にする. この液 145 mL にアセトニトリル 855 mL を加えて混合する.