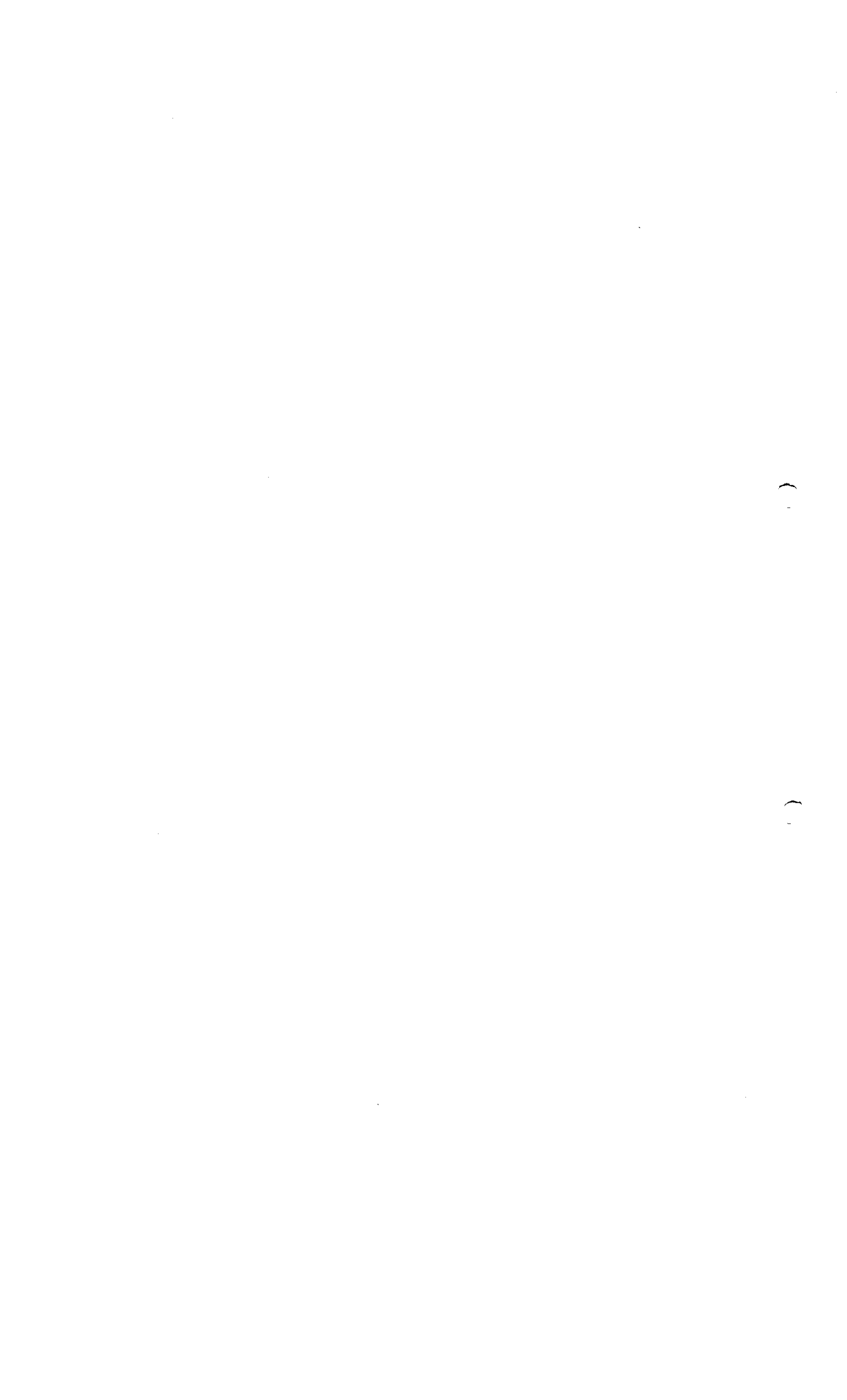


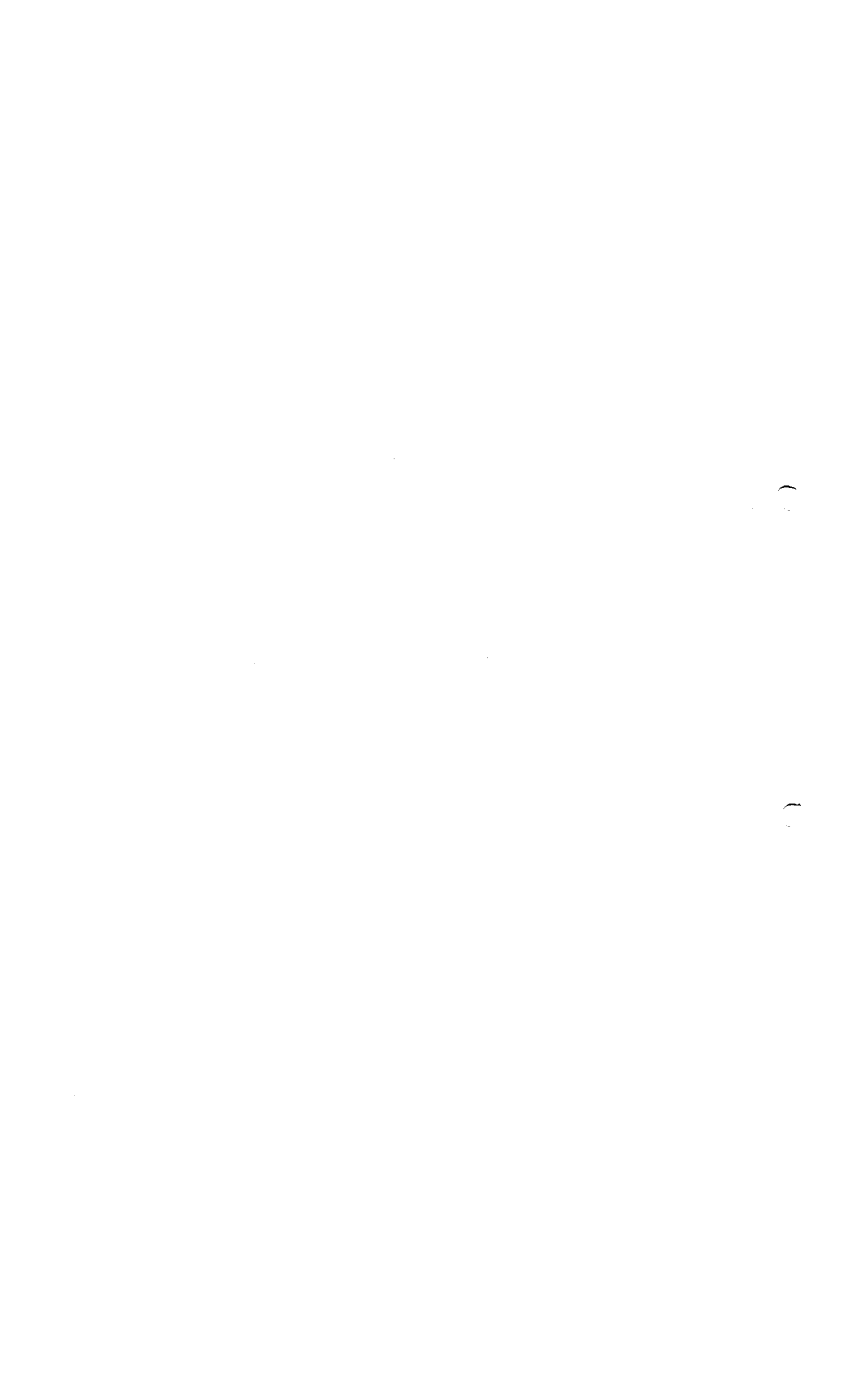
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Lista de abreviaturas: vea la sección 2.3 Resumen general de calidad, Introducción.

En el capítulo que aparece a continuación se presentan los estándares o materiales de referencia. Cuando se cuenta con ellos, se proporcionan ejemplos de los certificados de análisis.





1 PRP

1.1 Identificación de *Haemophilus*

El material de referencia es una vacuna conjugada liofilizada interna de *Haemophilus* a razón de 10 µg/vial (20 µg/mL tras la reconstitución de los viales con 0,5 mL de agua purificada), almacenada a +5 °C ± 3 °C.

1.2 Distribución del tamaño molecular

El material de referencia es un compuesto de moléculas de peso molecular conocido según lo establecido por La Farmacopea Europa (Ph. Eur.) 2.2.30 "Size Exclusion Chromatography", suministrado por un proveedor externo y que corresponde a ADN muy polimerizado de timo de ternero (Vt). Se conserva a +5 °C ± 3 °C. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

1.3 Contenido de ribosa

Tal como se define en la Ph. Eur. 2.5.31 "Ribose in Polysaccharide Vaccines", el material de referencia es un polvo de ribosa disponible a nivel comercial, que se conserva a temperatura ambiente. Esta solución la suministra un proveedor externo. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

1.4 Contenido de fósforo

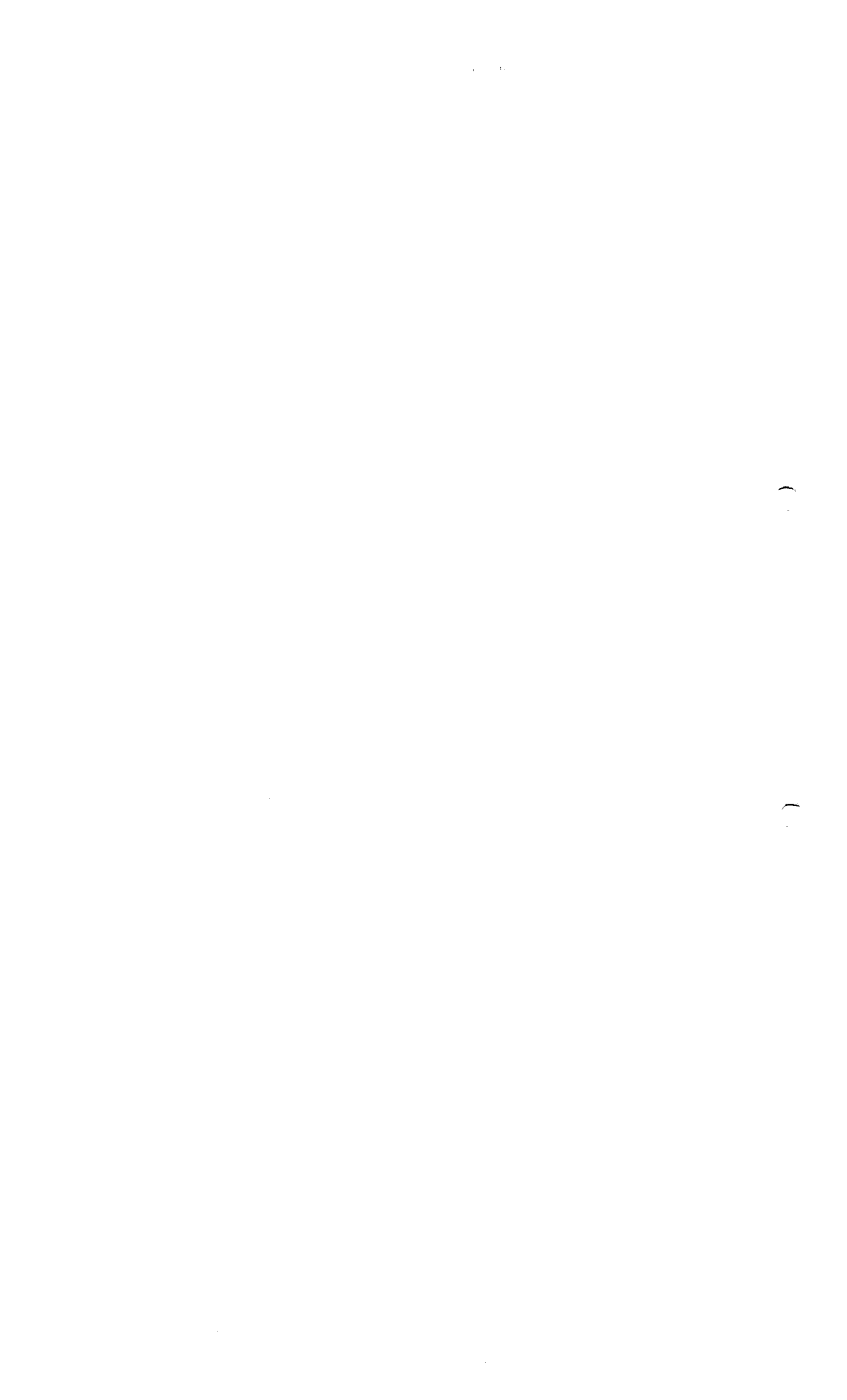
Tal como se define en la Ph. Eur. 2.5.18 "Phosphorus in Polysaccharide Vaccines", el material de referencia es un polvo desecado de dihidrogenofosfato de potasio (KH₂PO₄), que se conserva a temperatura ambiente. Es un compuesto disponible a nivel comercial y lo suministra un proveedor externo. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

1.5 Contenido proteico

El estándar de referencia es una solución de albúmina de suero bovino que se suministra con un contenido certificado y se adquiere del Instituto Nacional de Estándares y Tecnologías (NIST) o de otra dependencia oficial, se conserva a +5 °C ± 3 °C

Al final de esta sección se proporciona un ejemplo del certificado de análisis.





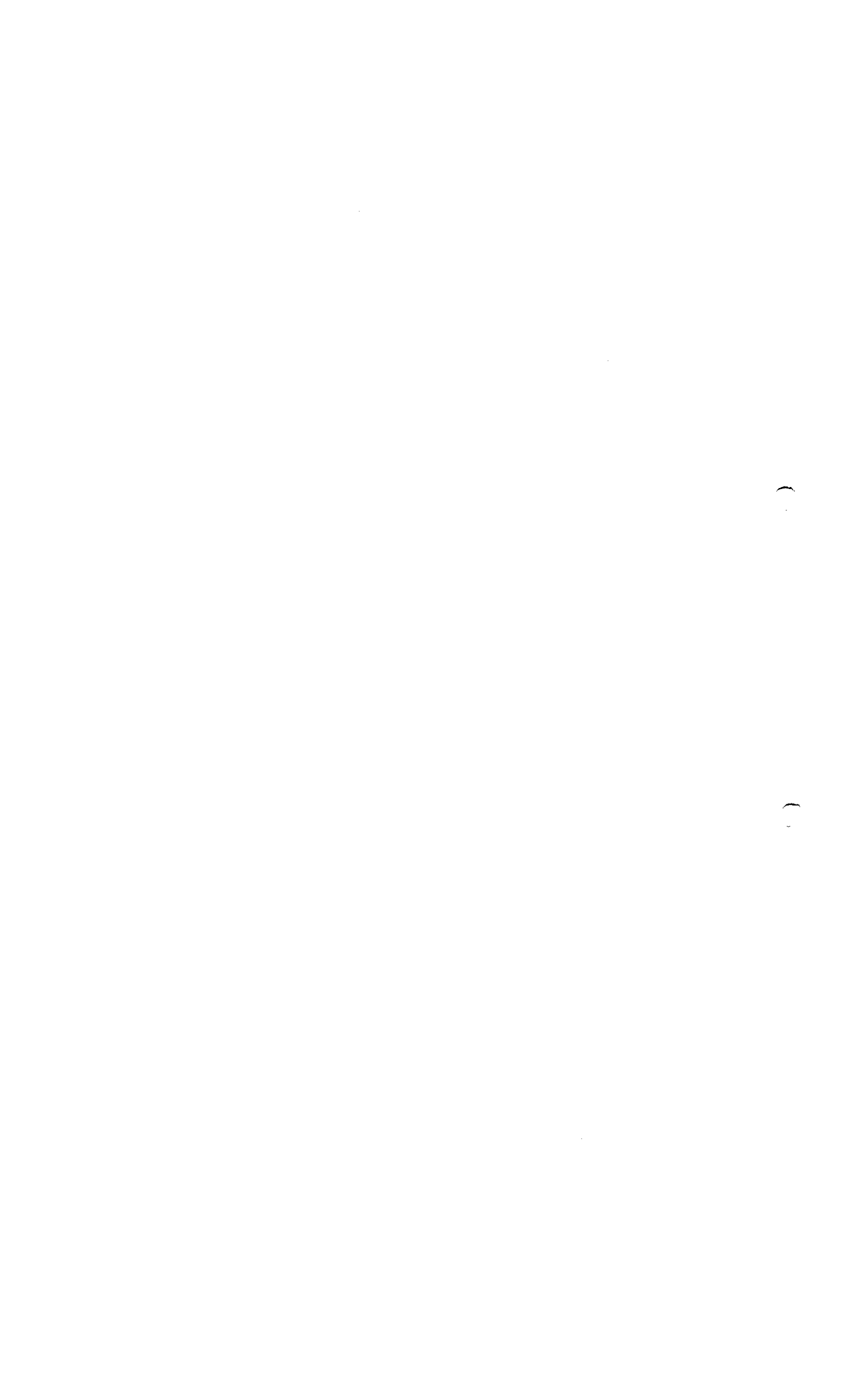
1.6 Contenido de endotoxinas bacterianas

El estándar de referencia utilizado para el análisis de endotoxinas bacterianas es parte de un kit de análisis suministrado por un proveedor externo. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Esta referencia, denominada endotoxina estándar de control (CSE), corresponde a un polvo liofilizado de endotoxinas de *E. Coli*. Las CSE fueron calibradas por el proveedor según la solución de endotoxina estándar de referencia (RSE proporcionada por la Dirección Europea de Calidad del Medicamento [EDQM]), según se requiere en la Ph. Eur. 2.6.14 "Bacterial Endotoxins". Sanofi Pasteur confirma la calibración de las CSE al momento de la entrega.

La CSE se suministra con un certificado de análisis que explica cómo realiza la comparación RSE/CSE el proveedor del kit. Este certificado también proporciona el volumen para reconstituir la referencia.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.





2 PRP-AH

2.1 Prueba límite de cianuros residuales

El material de referencia para cianuros es una solución de cianuro suministrada por un proveedor externo con un título de 1 g/L de cianuro en NaOH 0,1 M. Esta solución se conserva a $+5\text{ °C} \pm 3\text{ °C}$.

2.2 Contenido de fósforo

Vea el capítulo 1.4.

2.3 Contenido de ADH total y libre

El material de referencia es un polvo de ADH disponible a nivel comercial suministrado por un proveedor externo y que se conserva a $\leq -20\text{ °C}$. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

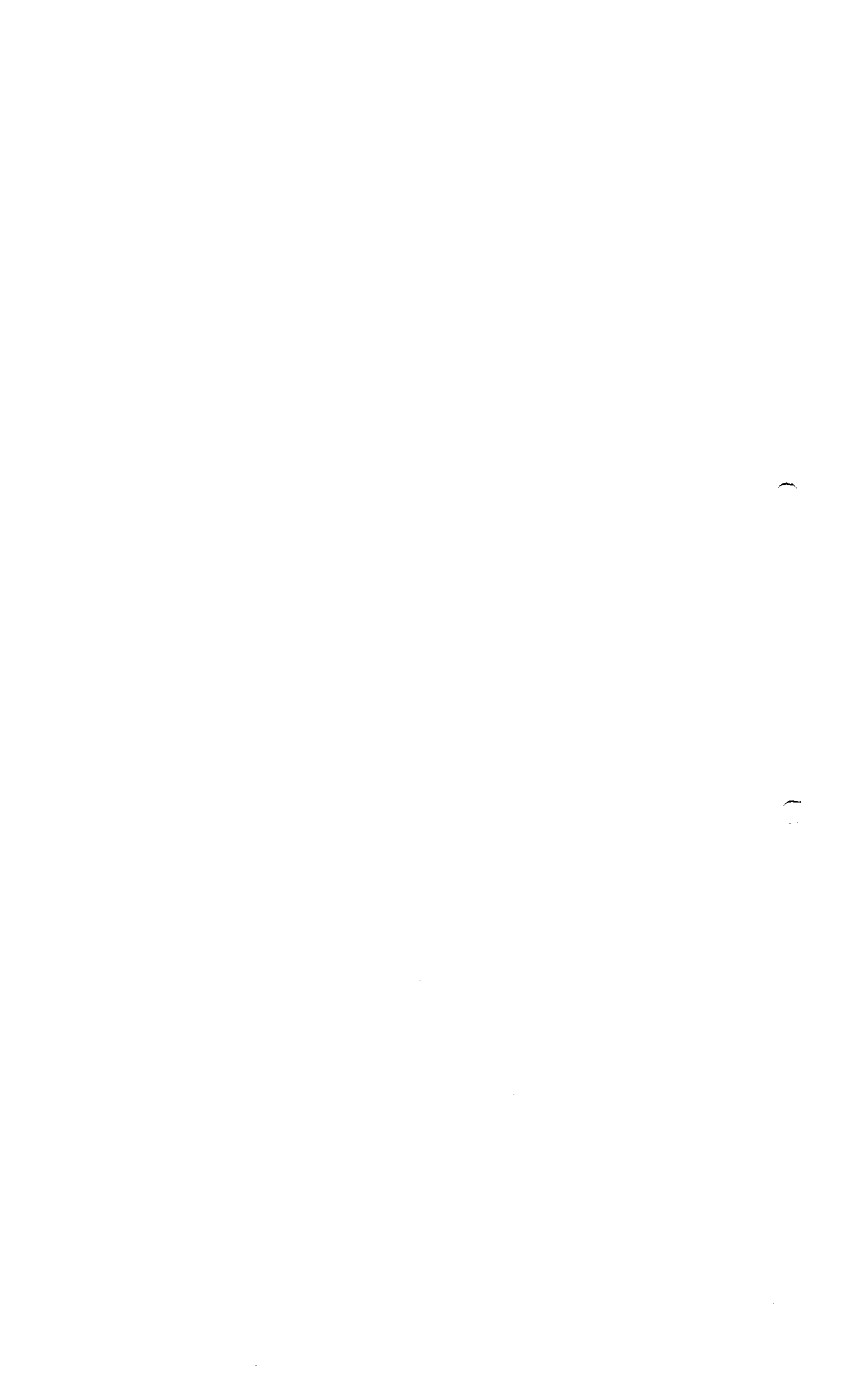
2.4 Distribución del tamaño molecular

El material de referencia es un compuesto de moléculas de peso molecular conocido según lo establecido por la Ph. Eur. 2.2.30 "Size Exclusion Chromatography", suministrada por un proveedor externo y que corresponde a ADN muy polimerizado de *Micrococcus lysodeikticus* (Vt). Se conserva a $+5\text{ °C} \pm 3\text{ °C}$. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

2.5 Identificación de *Haemophilus*

Vea el capítulo 1.1.





3 PTP

3.1 Título de floculación

El material de referencia es un estándar de floculación interno para el tétanos (antitoxina) calibrado de acuerdo con el estándar internacional de floculación para el tétanos (NIBSC) y almacenado a <-20 °C.

3.2 Distribución del tamaño molecular

El material de referencia es un kit con moléculas de peso molecular conocido según lo establecido por la Ph. Eur. 2.2.30 "Size Exclusion Chromatography", que contiene 0,05 % de dextrano azul (utilizado para determinar V_0) y 2 mg/mL de albúmina humana con acetiltriptofano (utilizado para determinar V_t), almacenado a $+5$ °C \pm 3 °C. Es un compuesto disponible a nivel comercial y lo suministra un proveedor externo. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

4 CTP

4.1 Contenido de fósforo

Vea el capítulo 1.4.

4.2 Contenido de formaldehído residual

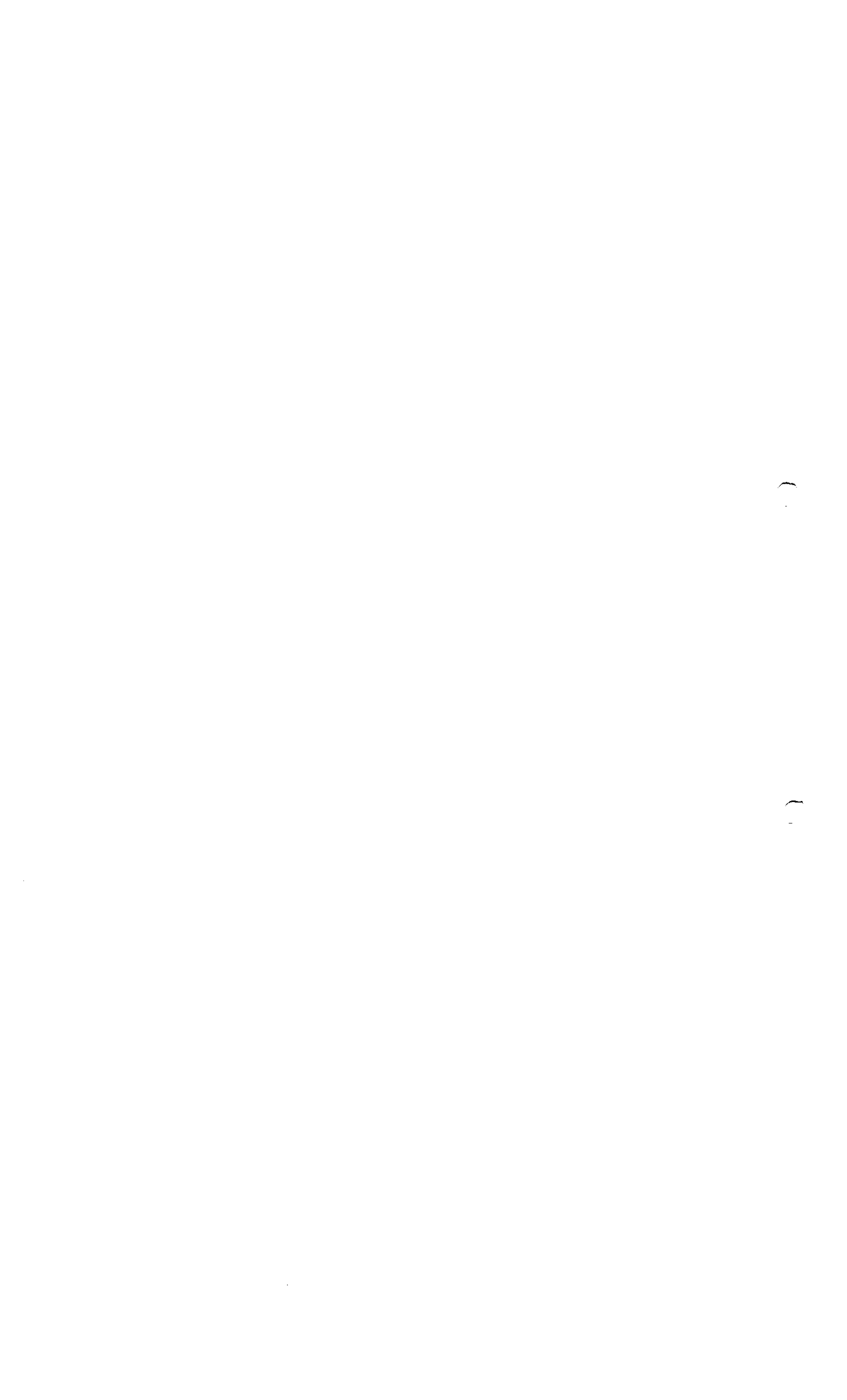
Tal como se describe en la Ph. Eur. 2.4.18 "Free Formaldehyde", el estándar de referencia del formaldehído es una solución de formaldehído p/p en una concentración del 34,5 % al 38,0 % disponible a nivel comercial, almacenada a temperatura ambiente. Esta solución la suministra un proveedor externo. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

El título de la solución se evalúa en el momento de su recepción (por titulación yodométrica) y se utiliza el título exacto expresado en mg/100 mL utilizando la densidad relativa.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

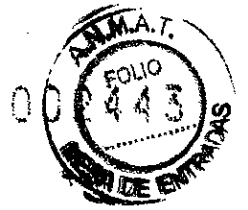
4.3 Título de floculación

Vea el capítulo 3.1.

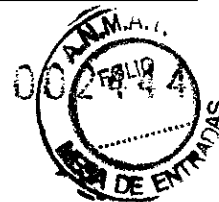


4.4 Distribución del tamaño molecular

Vea el capítulo 3.1.







5 PRP-T

5.1 Contenido de fósforo

Vea el capítulo 1.4.

5.2 Contenido proteico

Vea el capítulo 1.5.

5.3 Distribución del tamaño molecular

El material de referencia es un compuesto de moléculas de peso molecular conocido según lo establecido por la Ph. Eur. 2.2.30 "Size Exclusion Chromatography", suministrada por un proveedor externo y que corresponde a ADN muy polimerizado de timo de ternero (Vo). Esta solución se conserva a $+5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$.

Pullulan $\approx 110\text{ kDa}$ también se utiliza para la calibración. Este compuesto es un polímero de polisacáridos suministrado por un proveedor externo y corresponde a unidades de maltotriosa, también conocido como α -1,4- ; α -1,6-glucano. Se conserva a temperatura ambiente.

Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

5.4 Contenido de polisacáridos libres (PRP despolimerizado)

El material de referencia es un lote interno de PRP-AH de título conocido, que es conforme al momento de su liberación. Se conserva a $\leq -70\text{ }^{\circ}\text{C}$.

5.5 Contenido de proteína tetánica libre

Los materiales de referencia son un lote interno de PRP-T (almacenado $\leq -35\text{ }^{\circ}\text{C}$) y un lote interno de proteína tetánica (almacenado a $+5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$).

5.6 Prueba límite de EDAC y EDU residual

El material de referencia es un compuesto disponible a nivel comercial suministrado por un proveedor externo. Corresponde a 1-etil-3-(3-dimetilaminopropil) carbodiimida (EDAC). Se conserva a $\leq -20\text{ }^{\circ}\text{C}$. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.





5.7 Prueba límite de fenoles residuales

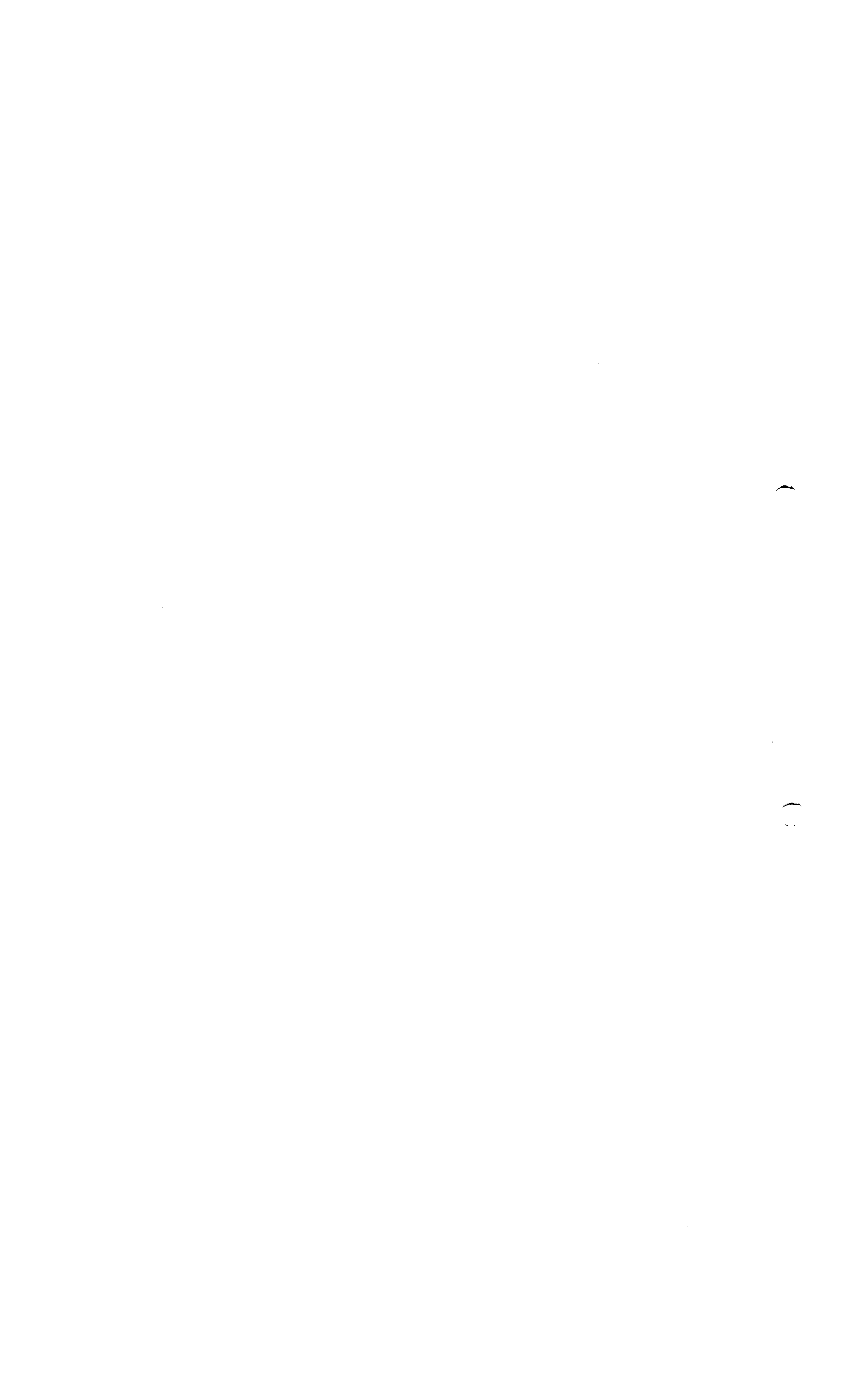
El material de referencia es una solución de fenol al 90%, conservada a temperatura ambiente. Esta solución la suministra un proveedor externo. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.

5.8 Contenido de sacarosa

El material de referencia es un polvo de sacarosa disponible comercialmente, conservado a temperatura ambiente. Sanofi pasteur puede usar otros proveedores si cuentan con una cualificación satisfactoria.

Al final de esta sección se proporciona un ejemplo del certificado de análisis.





3.2.S.5

Certificate of Analysis - DNA Calf Thymus - PRP-T


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SAMOPI PASTEUR S.A.


CHRISTIAN DOMINGUEZ
APODERADO
SAMOPI PASTEUR S.A.





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Product #



SIGMA-ALDRICH

Certificate of Analysis

- I
- D1
- Inf
- MS
- Sp
- Ce
- E
- Ce
- E
- Mc
- Pr
- Pr
- Bu
- As
- En
- Las
- D'

Product Name Deoxyribonucleic acid sodium salt from calf thymus, Type I, fibers
Product Number D1501
Product Brand Sigma
CAS Number 73049-39-5
Storage Temp 2-8°C

TEST	SPECIFICATION	LOT 105K7025 RESULTS
APPEARANCE	WHITE THREADS	CONFORMS
SOLUBILITY	REPORT RESULT	SLIGHTLY HAZY COLORLESS
RATIO A260/A280	NLT 1.8	1.9
A260 UNITS/MG SOLID	REPORT RESULT	16.7

UNIT DEFINITION
 ONE UNIT WILL YIELD AN A260 OF 1.0 IN 1.0ML OF 15MM SODIUM CHLORIDE AND 1.5MM SODIUM CITRATE, PH7.0 (1CM LIGHT PATH). ONE MG OF DNA IS EQUIVALENT TO APPROX. 20A260 UNITS.

ICP-ATOMIC EMISSION
 6 TO 13% SODIUM 6%
 5 TO 9% PHOSPHORUS 8%

PROTEIN BY LOWRY
 REPORT RESULT 5.6%

ENZYMATIC SUITABILITY
 TESTED AND FOUND SUITABLE AS A SUBSTRATE FOR DNASE SUITABLE

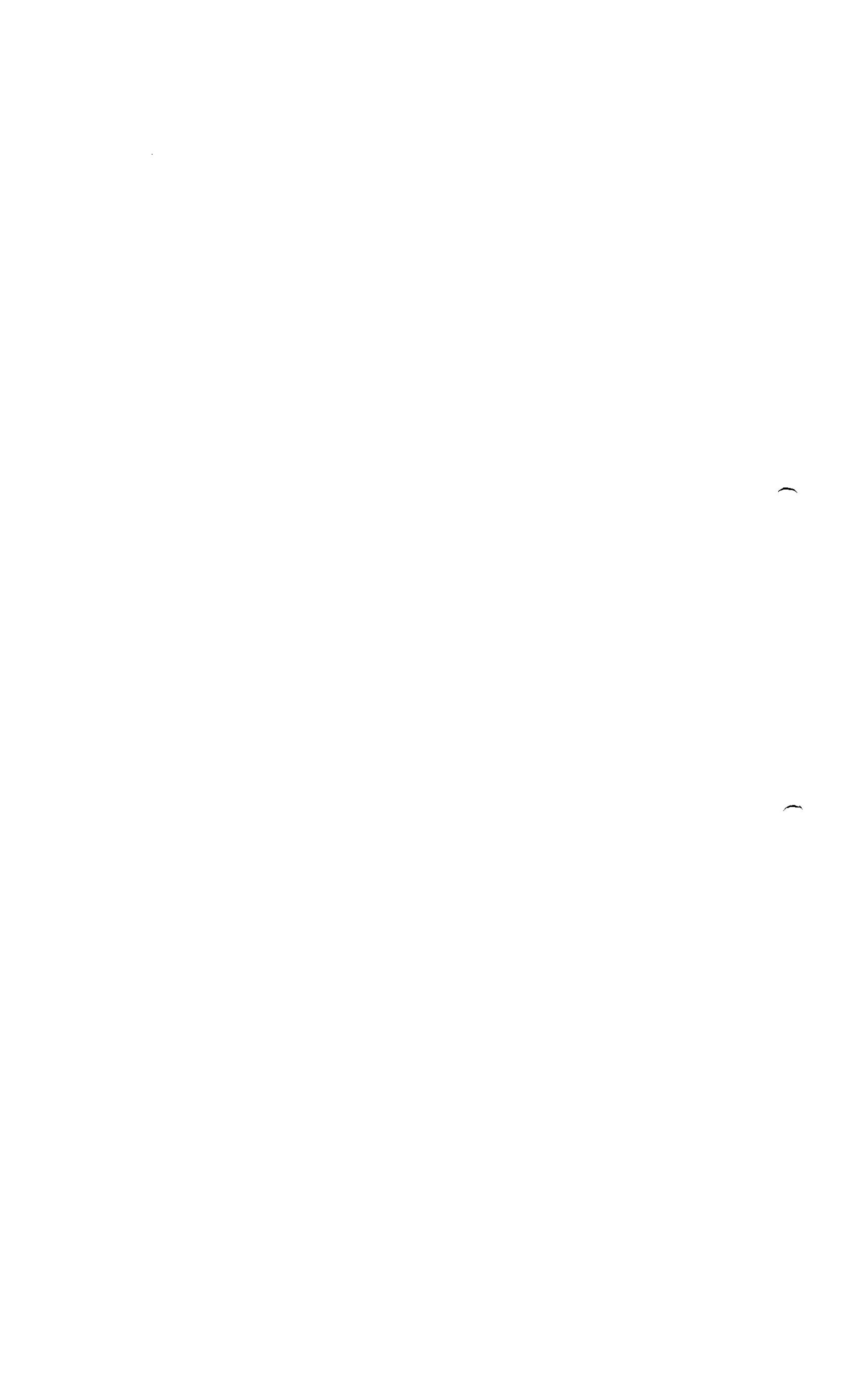
RECOMMENDED RETEST 4 YEARS MARCH 2010
QC ACCEPTANCE DATE MARCH 2006

Rodney Burbach, Supervisor
Analytical Services
St. Louis, Missouri USA

E. GRUA
28 JUN 2007

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CHRISTIAN DOMINGUEZ APODERADO
SANOFI PASTEUR S.A. SANOFI PASTEUR S.A.





3.2.S.5

Certificate of Analysis - Ribose - PRP-T


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOPI PASTEUR S.A.


CHRISTIAN DOMÍNGUEZ
APODERADO
SANOPI PASTEUR S.A.



Certificate of Analysis



<http://certificates.merck.de>

Date of print: 12.05.2006

1.07605.0000 D(-)-Ribose for biochemistry

Batch K31853905

Identity (IR-spectrum)	passes test	
Spec. rotation (α 20/D; 2 %; water)	-20.0	°
TLC-Test	conforms	
Water	0.3	%
Heavy metals (as Pb)	≤ 0.001	%

Test date (DD.MM.YYYY): 07.03.2003
Minimum shelf life (DD.MM.YYYY): 31.03.2008

Dr. Markus Schollmeier

responsible laboratory manager quality control

This document has been produced electronically and is valid without a signature

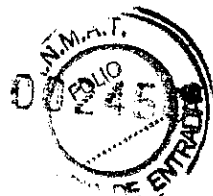
Merck KGaA 64271 Darmstadt Tel. (06151)72-0

SA-1.Aabr.12091246 905112 - 1010051000.000000 V. 057

ROXANA MONTEMILONE CHRISTIAN DOMINGUEZ
DIRECTORA TÉCNICA APODERADO
SANGUI PASTEUR S.A. SANGUI PASTEUR S.A.


Page 1 of 1




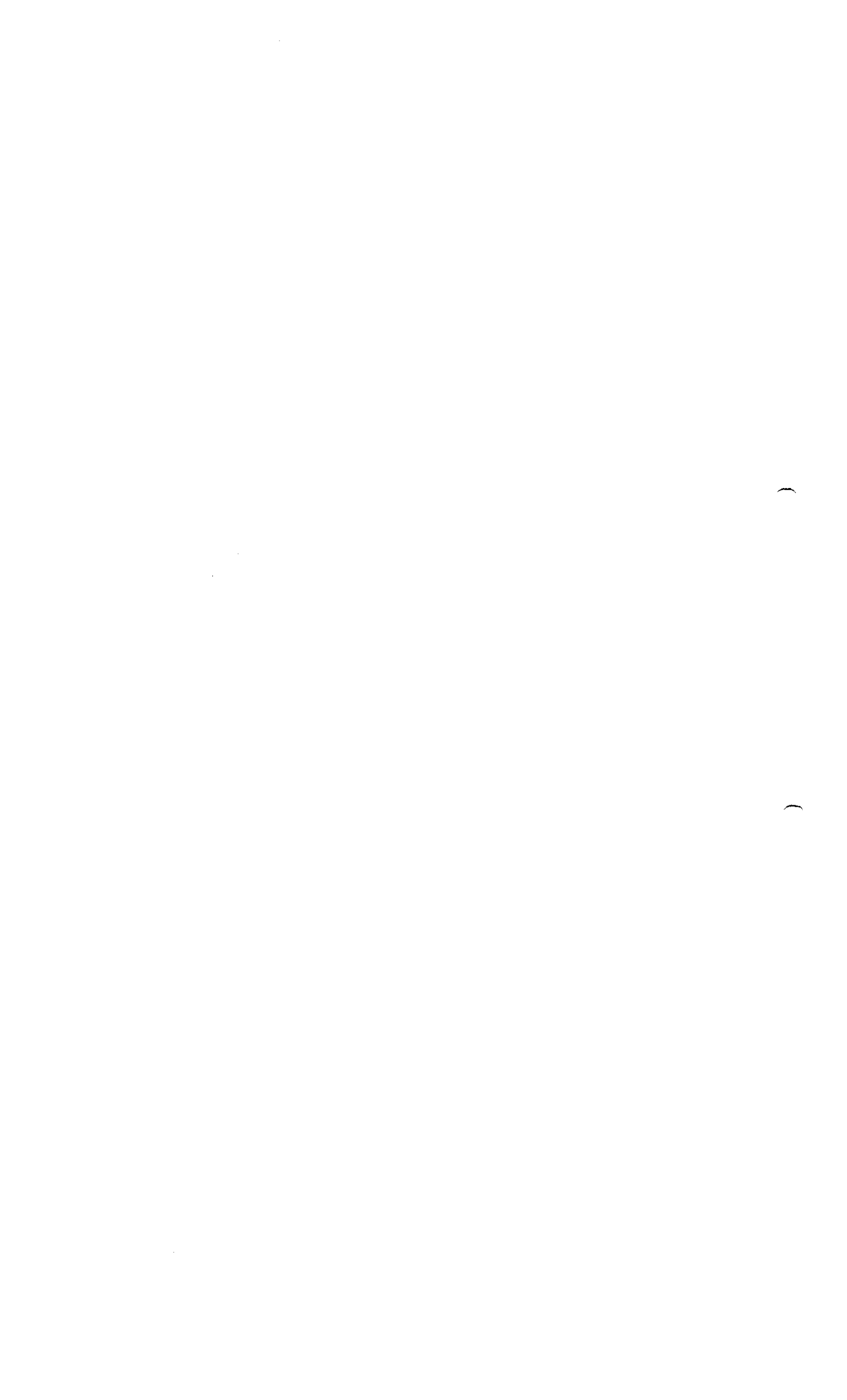


3.2.S.5

Certificate of Analysis - Potassium Dihydrogen Phosphate - PRP-T


ROXANA MONTEMILONE
DIRECTORA TÉCNICA
SANOFI PASTEUR S.A.


CHRISTIAN DOMINGUEZ
MODERADO
SANOFI PASTEUR S.A.



Bulletin d'analyse

Date d'edition: 08.11.2005

26936	Potassium dihydrogénophosphate NORMAPUR pour analyses
Lot	0506088

	Valeur mesurée	Valeurs spécifiées mini	Valeurs spécifiées maxi	
Titre	99.9	99.5		%
pH (c = 5 %, H ₂ O)	4.4	4.2	4.5	
Perte à la dessiccation (105°C)	< 0.2		0.2	%
Métaux lourds (en Pb)	< 10		10	ppm
SO ₄	< 30		30	ppm
Cl	< 5		5	ppm
Fe	< 10		10	ppm
N total	< 10			ppm
Na	< 150		150	ppm

Date de controle: 12.09.2005
A utiliser avant: 12.09.2010

Nous certifions que ce lot de produit est conforme aux spécifications ci-dessus.
BDL : En dessous de la limite de détection.

Didier Lagoutte Responsable Laboratoire d'analyse
Laboratoire d'Analyse Prolabo - Briare

Ce document est produit de maniere electronique et ne porte pas de signature.






3.2.S.5

Certificate of Analysis - Bovine Serum Albumin - PRP-T


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DIRECTORA TÉCNICA
SANOPI PASTEUR S.A.


CHRISTIAN DOMÍNGUEZ
ACUADERADO
SANOPI PASTEUR S.A.





National Institute of Standards & Technology



Certificate of Analysis

Standard Reference Material[®] 927d

Bovine Serum Albumin (7 % Solution)

(Total Protein Standard)

This Standard Reference Material (SRM) is intended primarily for use in the standardization of procedures employed in clinical analyses for total serum protein, for critical evaluation of daily working standards used in these procedures, and as a reference standard for assays of total protein by colorimetric methods. This SRM is a solution (mass fraction 7 %) of known protein concentration and purity. It conforms to the specification for standardized protein solution approved by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) [1]. The protein content of this SRM was determined using the biuret reference method [2] that is recommended for use in standardizing laboratory-prepared protein solutions and "normal" serum pools. Such standardized "normal" sera could then be used to calibrate refractometers or other instruments for serum protein estimations. SRM 927d may also be used for other procedures, such as gel diffusion, amino acid analysis, electrophoresis, nitrogen assays, or other tests that require well-characterized protein for calibration or evaluation. A unit of 927d consists of 10 ampoules, each containing 2.2 mL of solution.

In addition to the measurements using the biuret method, NIST made measurements of the bovine serum albumin (BSA) concentration using amino acid analysis. There is a discrepancy between the results from the two approaches. As the two different approaches are not determining the same measurand, it was decided to separately report the results from the two approaches as follows: 1) certified BSA concentration by amino acid analysis and 2) reference total protein concentration by the biuret method.

Certified Bovine Serum Albumin (BSA) Concentration and Uncertainty from Amino Acid Analysis: Two different methods of amino acid analysis were used to measure the concentration of bovine serum albumin (see "Analytical Methods" section). The certified results are shown in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST.

Reference Total Protein Concentration and Uncertainty as Determined Using the Biuret Method: The biuret reference method [2] was employed to determine protein concentration in SRM 927d using SRM 927c as an external standard. The reference protein concentration and uncertainty are shown in Table 2. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Additional Reference Values and Uncertainties: Reference values are provided in Table 3 for additional properties including fill volume, pH, density, absorbances at various wavelengths, and relative molecular mass as determined using electrospray ionization mass spectrometry.

Expiration of Certification: This certification is valid until 30 September 2010, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification will be nullified if the SRM is contaminated or modified.

Stephen A. Wise, Chief
Analytical Chemistry Division

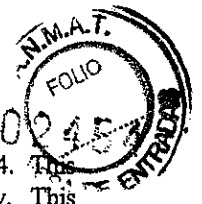
Gaithersburg, MD 20899
Certificate Issue Date: 02 February 2006

Robert L. Watters, Jr., Chief
Measurement Services Division

SRM 927d

ROXANA MONTEMILONE CHRISTIAN DOMINGUEZ
DIRECTORA TÉCNICA PRODEBAC 1 of 5
SANOFI PASTEUR S.A. SANOFI PASTEUR S.A.





Information Value: A literature value for the optical absorbance of bovine serum albumin is given in Table 4. This is a noncertified value with no reported uncertainty as there is insufficient information to assess uncertainty. This information value is given to provide additional characterization of the material.

The overall direction and coordination of technical measurements leading to the certification were performed by M.J. Welch and D.M. Bunk of the NIST Analytical Chemistry Division.

Analyses were performed by D.M. Bunk, L.T. Sniegowski, and M. Vergne of the NIST Analytical Chemistry Division.

The statistical analysis of the data used for certification was performed by N.F. Zhang of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

NOTICE AND WARNING TO USERS

SRM 927d IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. The blood used in the preparation of SRM 927d Bovine Serum Albumin (7 % Solution) was collected from cattle sourced in the United States. Only blood from cattle/carcasses that have passed ante-mortem and post-mortem USDA-Food Safety Inspection Service (FSIS) inspection was used. This material was collected prior to any known case of Bovine Spongiform Encephalopathy (BSE) in the United States. There were no additives to the pooled serum prior to protein purification.

INSTRUCTIONS FOR USE

Storage: This SRM is supplied to the user in sealed ampoules. The SRM should be stored in a refrigerator at a temperature between 2 °C and 8 °C. The ampoules should not be frozen because of possible breakage of ampoules during the thawing process. Once an ampoule is opened, the solution should be used promptly. Any unused solution in opened ampoules should be discarded.

Inappropriate Uses: This SRM is not intended to be used as a standard for dye-binding tests, for checking precalibrated refractometers, for immunochemical methods, or as an additive for bilirubin standardization.

SOURCE, PREPARATION, AND ANALYSIS¹

Source of Material: The bovine serum albumin solution was prepared by Bionostics Inc., (Acton, MA, USA) under contract with Bioreclamation, Inc., (Hicksville, NY, USA). The bovine serum was produced for manufacture into products for pharmaceutical use by West Laboratories, Inc. at USDA EST. #245-J, Iowa Beef Packers, Inc., (Joslin, IL, USA).

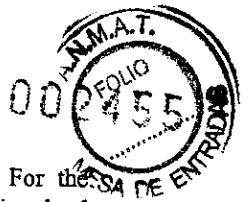
The BSA for this SRM was dissolved in 0.02 mol/L sodium chloride and the pH adjusted to 6.5 - 6.8 with 1.0 mol/L sodium hydroxide. The material was sterilized by membrane filtration and tested for sterility by approved methods [3].

Preparation of Dilutions: Protein solutions of lower concentration may be prepared by transferring the appropriate aliquot to a volumetric flask and diluting to volume. Diluents are not furnished with the SRM; however, an aqueous sodium chloride diluent, such as a solution having a concentration of 0.15 mol/L, may be used.

¹Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

[Handwritten signature]
ROXANA MONTEALON
DIRECTORA TECNICA
SANOFI PASTEUR S.A.
CRISTIAN ALONSO
MODERADO
SANOFI PASTEUR S.A.





Analytical Methods: All analyses for the certified and reference values were performed at NIST. For the determination of BSA, two different methods for determining amino acids were used. The first method involved isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS). Samples of SRM 927d and 927c (as a control) were hydrolyzed with vapor phase hydrochloric acid (HCl) for 2 h at 150° C in sealed vials. After hydrolysis, the samples were lyophilized and then reconstituted with 0.2 % formic acid in acetonitrile containing isotope labeled analogs of phenylalanine, proline, and valine. Amino acids were separated using hydrophilic interaction chromatography (HILIC) on a polyhydroxyethyl aspartate column with gradient elution. Measurements were performed on a triple quadrupole mass spectrometer, monitoring specific transitions for each amino acid. The measurements were calibrated with different dilutions of SRM 2389 *Amino Acids in 0.1 mol/L HCl*. Data were collected for phenylalanine, proline, and valine. Based upon the known amino acid sequence for BSA, the concentration of BSA was calculated from the concentrations determined for each of the amino acids. For the second method, a commercial amino acid analyzer was used following hydrolysis as described above. This analyzer uses an ion exchange resin column to separate the amino acids, followed by derivatization with ninhydrin, and detection by spectrophotometry at 570 nm and 440 nm. These measurements were calibrated with dilutions of SRM 2389. The amino acids used for quantitation were alanine, leucine, lysine, and aspartic acid/asparagine.

The reference total protein concentration was measured using the biuret reference method for total serum protein [2]. The measurements involve a direct comparison between the current SRM lot (927d) and the previous lot (927c) and were performed on a Varian Cary 219 spectrophotometer.

The reference values for various properties determined for SRM 927d are given in Table 3. Absorbances were measured in accordance with requirements specified for a standard BSA solution [1]. Measurement of the pH was performed using an Orion Model 501 pH meter with a glass body combination pH electrode calibrated with pH 4 and pH 7 buffers. Density was measured using the Lang-Levey pipet method [4]. Fill masses were determined gravimetrically and fill volumes were calculated from the fill masses and mean density.

Relative molecular mass was determined using liquid chromatography/mass spectrometry (LC/MS). Measurements were performed on a single quadrupole mass spectrometer operated in the positive ion mode and coupled to a capillary LC with a commercial C-4 column, 0.5 mm x 50 mm, held at 40 °C. Gradient elution using 0.05 % trifluoroacetic acid in water and in acetonitrile was used. Horse apomyoglobin was used for mass calibration of the mass spectrometer. The molecular masses of the seven major forms of BSA found in SRM 927d are shown in Table 3 in decreasing order of abundance. The previous lot (SRM 927c) had a similar range of molecular masses.

SRM 927d


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