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Datasheet for the decision of 19 March 2026

Case Number: T 0461/24 - 3.3.04

Application Number: 16757608.1

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Language of the proceedings: EN

Title of invention:
Method for the reduction of host cell proteins in affinity chromatography

Patent Proprietor:
F. Hoffmann-La Roche AG
GENENTECH, INC.

Opponents:
Maiwald GmbH
Withers & Rogers LLP
Elkington and Fife LLP
WALLINGER RICKER SCHLOTTER TOSTMANN

Headword:
Protein A chromatography/ROCHE GENENTECH

Relevant legal provisions:

EPC Art. 56, 83, 123(2)
RPBA 2020 Art. 12(4)

Keyword:

Main request - inventive step (no)
Auxiliary request - amendments - allowable (yes) - sufficiency of disclosure (yes) - inventive step (yes)
Amendment to case - amendment within meaning of Art. 12(4) RPBA - complexity of amendment - admitted into the proceedings (no)

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Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0

Case Number: T 0461/24 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 19 March 2026

Appellant I: (Patent Proprietor 1)	F. Hoffmann-La Roche AG Grenzacherstrasse 124 4070 Basel (CH)
Appellant I: (Patent Proprietor 2)	GENENTECH, INC. 1 DNA Way South San Francisco, CA 94080 (US)
Representative:	Vossius & Partner Patentanwälte Rechtsanwälte mbB Siebertstraße 3 81675 München (DE)
Appellant II: (Opponent 1)	Maiwald GmbH Elisenhof, Elisenstrasse 3 80335 München (DE)
Representative:	Maiwald GmbH Elisenhof Elisenstraße 3 80335 München (DE)
Respondent: (Opponent 2)	Withers & Rogers LLP 2 London Bridge London SE1 9RA (GB)
Representative:	Tombling, Adrian George Withers & Rogers LLP 2 London Bridge London SE1 9RA (GB)
Respondent: (Opponent 3)	Elkington and Fife LLP Prospect House 8 Pembroke Road Sevenoaks Kent TN 13 1XR (GB)

Representative:	Elkington and Fife LLP Prospect House 8 Pembroke Road Sevenoaks, Kent TN13 1XR (GB)
Respondent: (Opponent 4)	WALLINGER RICKER SCHLOTTER TOSTMANN Patent- und Rechtsanwälte Partnerschaft mbB Zweibrückenstrasse 5-7 80331 Munich (DE)
Representative:	Wallinger Ricker Schlotter Tostmann Patent- und Rechtsanwälte mbB Zweibrückenstraße 5-7 80331 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted/ electronically transmitted on 5 February 2024 concerning maintenance of the European Patent No. 3337812 in amended fo rm.**

Composition of the Board:

Chairwoman	M. Pregetter
Members:	D. Luis Alves
	R. Romandini

Summary of Facts and Submissions

I. European patent No. 3 337 812, entitled "*Method for the reduction of host cell proteins in affinity chromatography*", was granted on European patent application No. 16 757 608.1, filed as an international application published as WO 2017/032686 (in the following, the application as filed).

II. Four oppositions were filed, invoking the grounds for opposition under Article 100(a) EPC, lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC), as well as the grounds under Article 100(b) EPC.

III. The opposition division decided that, account being taken of the amendments in the form of auxiliary request 10, the patent and the invention to which it related met the requirements of the EPC (Article 101(3)(a) EPC).

IV. The patent proprietors (appellant I) and opponent 1 (appellant II) both filed appeals against that decision. Opponents 2, 3 and 4 are respondents to the patent proprietors' appeal.

V. With its statement setting out the grounds of appeal, appellant I filed sets of claims of auxiliary requests 1 to 13.

VI. With its statement setting out the grounds of appeal, appellant II submitted arguments to the effect that the request as held allowable by the opposition division did not comply with the requirements of

Articles 123(2), 83 and 56 EPC. Documents D52 to D54 were filed.

VII. Appellant I submitted a reply to appellant II's statement of grounds of appeal.

Appellant II submitted a reply to appellant I's statement of grounds of appeal.

Appellant II filed further arguments in the letter dated 30 April 2025, addressing the admittance of documents D53 and D54 into the appeal proceedings.

VIII. Opponent 3 (respondent) submitted a reply to appellant I's appeal. Opponents 2 and 4 made no submissions in appeal proceedings.

IX. The board appointed oral proceedings and in a communication pursuant to Article 15(1) RPBA informed the parties of its preliminary opinion on some of the issues in the appeal.

X. Oral proceedings were held in the absence of opponent 2 and opponent 4, as announced previously with the letters dated 23 February 2026 and 19 February 2026, respectively. At the oral proceedings, appellant I made auxiliary request 6 its main request and auxiliary request 7 (claims held allowable by the opposition division) its auxiliary request 1. All other claim requests were withdrawn.

XI. Claim 1 of the **main request** reads as follows.

"1. Use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the

protein A chromatography is used to purify a human IgG4 or IgG1 isotype antibody, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less, wherein the host cell protein is phospholipase B-like 2 (PLBL2), wherein the low conductivity aqueous solution
(i) comprises about 0.1 mM to about 8 mM Tris,
(ii) comprises about 0.05 mM to about 2 mM potassium phosphate, or
(iii) is deionized water."

Auxiliary request 1 consists of 14 claims. Claims 1, 7 and 8 are independent claims. Claim 1 differs from claim 1 of the main request in that alternatives (i) and (ii) have been deleted. Claims 1, 7 and 8 read as follows.

"1. Use of a low conductivity aqueous solution in a wash step of a protein A chromatography for reducing the content of a host cell protein wherein the protein A chromatography is used to purify a human IgG4 or IgG1 isotype antibody, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less, wherein the host cell protein is phospholipase B-like 2 (PLBL2), wherein the low conductivity aqueous solution is deionized water.

7. Method for producing a human IgG4 or IgG1 isotype antibody comprising the steps of
a) cultivating a cell comprising a nucleic acid encoding a human IgG4 or IgG1 isotype antibody,
b) recovering the human IgG4 or IgG1 isotype antibody from the cell or the cultivation medium,
c) contacting the human IgG4 or IgG1 isotype antibody with a protein A chromatography material,

d) washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less, wherein the amount of a host cell protein is reduced and wherein said host cell protein is phospholipase B-like 2 (PLBL2),
e) recovering the human IgG4 or IgG1 isotype antibody from the protein A chromatography material
and thereby producing the human IgG4 or IgG1 isotype antibody
wherein the low conductivity aqueous solution is deionized water.

8. Method for purifying a human IgG4 or IgG1 isotype antibody from a sample comprising the steps of
a) providing a sample comprising a human lgG4 or IgG1 isotype antibody,
b) purifying the human IgG4 or IgG1 isotype antibody with a protein A chromatography method/step, comprising washing the protein A chromatography material with a low conductivity aqueous solution, wherein the low conductivity aqueous solution has a conductivity value of about 0.5 mS/cm or less, wherein the amount of a host cell protein is reduced and wherein said host cell protein is phospholipase B-like 2 (PLBL2),
wherein the low conductivity aqueous solution is deionized water."

XII. The following documents are referred to in this decision:

D5: Vanderlaan, M., presentation "Recent experiences with Host Cell Protein Impurity Analysis" dated 13 November 2014

D6: Vanderlaan, M. *et al.*, BioProcess International 13(4), April 2015, pages 18-29, 59

D7: WO 2014/207763 A1

D8: WO 2011/038894 A1

D9: EP 2583973 A1

D11: WO 2015/038888 A1

D13: Tran, B. *et al.*, Journal of Chromatography A, 1438, 2016, pages 31-38

D14: US 2006/0142549 A1

D16: Shukla, A.A. and Hinckley, P., Biotechnology Progress 24, 2008, pages 1115-1121

D26: Ishihara, T. and Hosono, M., Journal of Chromatography B, 995-996, May 2015, pages 107-114

D27: Hogwood, C.E.M. et al., Current Opinion in Biotechnology 30, 2014, pages 153-160

D30: GE Healthcare, "Hydrophobic Interaction and Reversed Phase Chromatography", Handbook, GE Healthcare 2006

D53: Elin Monié, Master's degree project: "Evaluation of the 96-well format for screening of chromatographic buffer conditions", 2006

XIII. Appellant I's arguments relevant to this decision may be summarised as follows.

Admittance of document D53 into the appeal proceedings

This document was late filed and should not be admitted into the appeal proceedings. It was filed to raise inventive-step objections against the embodiment using deionised water in claim 1 of the main request and claim 1 of auxiliary request 1. However, claim 1 of the main request was identical to claim 1 of auxiliary request 2 filed with the reply to the notices of opposition, and claim 1 of auxiliary request 1 was identical to claim 1 of auxiliary request 10 filed on the final date set under Rule 116 EPC. There was therefore no justification for filing this document in appeal proceedings only.

Moreover, the document was not *prima facie* relevant as it did not mention the host cell protein (HCP) PLBL2, and it was not possible to correlate PLBL2 with total HCP levels. Moreover, deionised water was only used with a cell lysate that did not contain any antibody to be purified. Its use was therefore not directed to the purification of an antibody.

Main request
Inventive step (Article 56 EPC)

The closest prior art was represented by document D11.

The patent showed the testing of low-conductivity solutions ranging from deionised water to 6 mM Tris at pH 8.0. Each of these wash solutions resulted in improved removal of PLBL2 relative to the control or to the load.

Accordingly, the problem to be solved in view of the disclosure in document D11 was the provision of an improved Protein A wash step for PLBL2 removal.

The solution defined in claim 1 was not obvious. The prior art did not disclose the features of the wash solutions (i), (ii) or (iii) in claim 1. Neither did any of D7, D8, D9 and D11 provide a general teaching to use low-conductivity wash solutions. It was disputed that document D9 disclosed two low-conductivity wash solutions since the conductivity value for 10 mM Tris did not fall within the range specified in claim 1. Thus, only one of the buffers disclosed in this document was a low-conductivity buffer according to claim 1. Further, document D9 did not disclose PLBL2.

Even if the objective technical problem were formulated as the provision of an alternative, the claimed solution was not obvious because the wash solutions in claim 1 were not in the toolbox of the skilled person.

Auxiliary request 1
Inventive step (Article 56 EPC)

Document D9 did not represent the closest prior art since it did not disclose removal of PLBL2.

Deionised water resulted in an increased reduction in PLBL2 compared to the reduction obtained in document D11. This could be seen from a comparison of Example 7A in the patent with document D11 since Example 7A used a potassium buffer as the high-conductivity wash solution and could therefore correspond to the high-salt wash buffer in document D11. The comparison with wash

solutions b and c in paragraph [0148] of the patent was not relevant.

None of the cited documents disclosed the use of deionised water. Therefore, the solution defined in claim 1 was not obvious.

Amendments (Article 123(2) EPC)

The feature "deionized water" was disclosed in the application as filed in the following passages: items 148 and 149 of the section "Specific embodiments of the invention" and in the paragraph bridging pages 8 and 9.

The feature PLBL2 represented a single selection from two alternatives for the preferred HCP, i.e. PLBL2 and clusterin.

Disclosure of the invention (Article 83 EPC)

Example 7 of the patent showed a reduction of PLBL2 for six antibody preparations using a wash step with deionised water, including four antibodies of the IgG1 subtype and two of the IgG4 subtype. In all cases, a very significant reduction compared to the load solution was achieved. In almost all cases, washing with deionised water resulted in a superior removal when compared to other wash solutions.

Document D13 did not use the low-conductivity buffers defined in the patent. Therefore, this document could not provide evidence that the claimed invention could not be implemented for all IgG1 and IgG4 antibodies.

Appellant II did not provide any evidence that the use of deionised water did not result in a purification as claimed.

XIV. Appellant II's arguments relevant to this decision may be summarised as follows.

Admittance of document D53 into the appeal proceedings

The first opportunity to file this document was with the statement of grounds of appeal. An opponent could not be required to provide arguments for every alternative in an auxiliary request. Therefore, the opponent could not have been required to provide arguments for the embodiment using deionised water, which was only one of the alternatives in auxiliary request 2 filed with the reply to the notices of opposition. This embodiment was not present in the claims as granted, and it was mentioned only once in the description of the patent. Moreover, in opposition proceedings, then auxiliary request 10 was filed only at the latest date possible under Rule 116 EPC, so the opponent had only two months to file this document in reply.

The document was *prima facie* relevant. The fact that it did not mention PLBL2 did not affect its relevance. It was also not of relevance whether it showed separation of an antibody from HCP.
Furthermore, it was straightforward to analyse because the only relevant question was whether it included deionised water as a buffer.

Main request
Inventive step (Article 56 EPC)

The difference between the use defined in claim 1 and the disclosure in document D11 was a low-conductivity wash solution versus a high-conductivity wash solution. However, low conductivity did not result in improved PLBL2 reduction. Rather, based on the results in the patent, a low-conductivity wash solution resulted in a poorer reduction.

It was not necessary that the characteristics of the wash solutions (i) to (iii) in claim 1 be disclosed in a document for the claimed use to be considered obvious (see T 1426/23, Reasons 5.3). In fact, those characteristics were completely arbitrary. To the contrary, it was sufficient that the technical feature common to the three alternatives claimed was mentioned. This was the low conductivity of the wash solution. Documents D7 to D9 disclosed a wide range of washing solutions effective at removing PLBL2 at least to some extent (see D7, wash solution with conductivity as low as 1 mS/cm; D8, 5 mM Tris and 5mM sodium chloride; D9, 10 mM Tris at pH 7.0). Document D11 disclosed the routine testing of various aqueous wash solutions, including various compositions and pH. A variation of pH could affect the conductivity.

Auxiliary request 1
Inventive step (Article 56 EPC)

Any of documents D7 to D9, D11, D26 and D53 could be taken as the starting point for the assessment of inventive step.

Regardless of whether claim 1 specified deionised water or an alternative wash solution, the technical information in the claim remained, as was the case for claim 1 of the main request, to use a low-conductivity wash solution. This, however, was already known from documents D8 and D9 and did not involve an inventive step.

Document D9 taught that a low-conductivity wash solution could be used. Document D26 used 10 mM histidine, which was a low-conductivity solution. While PLBL2 was not mentioned, it was a major HCP the skilled person would aim to remove.

Example 7 of the patent did not compare deionised water with a low-conductivity histidine buffer. Without such a comparison, no technical effect of deionised water could be acknowledged. Moreover, Example 7E showed that deionised water performed worse than wash solutions b and c, which were to be considered low conductivity wash solutions, based on the examples given in paragraph [0056] of the patent (solutions with a pH 8.5 or higher and 55 mM Tris or 30 mM Tris were given as examples of low-conductivity wash solutions).

The objective technical problem when starting from the disclosure in document D9 was therefore the provision of an alternative wash solution for Protein A chromatography. However, it was common general knowledge that deionised water has low conductivity. The use of an alternative, for which no advantage had been shown, did not involve an inventive step.

Document D11 disclosed that PLBL2 could be removed in a hydrophobic interaction chromatography step. The use defined in claim 1 differed in that PLBL2 bound to the

antibody was removed using deionised water as the wash solution during Protein A chromatography in addition, or instead of, hydrophobic interaction chromatography. No improvement was associated with this difference. Therefore, the objective technical problem was the provision of an alternative Protein A wash step for PLBL2 removal. Since document D11 taught that PLBL2 could be removed by hydrophobic interaction chromatography, the skilled person would realise that PLBL2 bound to the antibody via hydrophobic interactions. These were known from document D30 to be reversible. It was common general knowledge that high salt concentrations promote hydrophobic interactions, whereas low salt concentrations inhibit them. The skilled person would have applied this teaching to Protein A chromatography and expect that lowering the conductivity in a wash step would remove contaminants that bind via hydrophobic interactions, such as PLBL2. Both D7 and D14 disclosed low-conductivity and high-conductivity washes. Therefore, the claimed use was obvious in view of the common general knowledge or the disclosure in document D6.

Amendments (Article 123(2) EPC)

Claim 1 did not comply with the requirements of Article 123(2) EPC because the application as filed did not disclose the feature "deionized water" in combination with the further features of claim 1.

The application as filed taught that deionised water was not a suitable wash solution or was suitable only in some applications, which, however, were not specified (see application as filed, paragraph bridging pages 9 and 10). Thus, deionised water was not

disclosed as a general embodiment in the application as filed.

Moreover, claim 1 combined this feature with PLBL2. Only Example 7 mentioned both. However, Example 7 could not serve as a basis for claim 1 since it included further features not present in the claim.

Disclosure of the invention (Article 83 EPC)

For the requirements of sufficiency of disclosure to be met, the removal of PLBL2 should be achieved with deionised water in combination with any IgG4 or IgG1 antibody, with deionised water as the sole wash, and with deionised water in combination with any preceding or subsequent wash solutions and elution buffer.

However, all the examples in experiment 2 used a low-conductivity wash preceded by a sequence of an intermediate-conductivity wash followed by a high-conductivity wash and again an intermediate-conductivity wash, and followed by elution with a high-conductivity wash. The application did not show PLBL2 removal in a different setting. This was in agreement with the sequence of wash and elution steps as described in documents D7 and D14. Therefore, the broad use as defined in claim 1 was not sufficiently disclosed. Similar considerations applied to Example 6.

Further, the application did not include any data demonstrating PLBL2 removal with a single wash step. In all experiments using deionised water as the wash solution, this was immediately preceded by a high-conductivity wash and immediately followed by elution at high conductivity.

Example 7 did not show PLBL2 removal by deionised water. It was not plausible that using deionised water as the single wash step between load and elution would reduce PLBL2 to the level required for therapeutic antibodies.

In view of the explicit warning in paragraph [0051] of the patent that deionised water is not suitable for some applications, claim 1 included non-working embodiments. However, the application contained no teaching as to the circumstances under which deionised water was suitable or unsuitable.

The reduction in PLBL2 was highly dependent on the antibody to be purified. Not only the antibody isotype but also its amino acid sequence impacted the reduction (see documents D5, D6, D13 and D27). Therefore, it was not credible that the use of deionised water would result in PLBL2 reduction over all the embodiments claimed.

XV. The arguments of the respondent-opponent 3, relevant to this decision, may be summarised as follows.

Main request
Inventive step (Article 56 EPC)

Documents D7 to D9 and D11 could each be considered the closest prior art. Document D7 disclosed wash solutions of conductivity in the range of 1 to 3 mS/cm. Document D8 disclosed various aqueous wash solutions, including a solution of 20 mM Tris and 20mM sodium chloride at pH 7.0. This was a much lower concentration than that considered to be of high conductivity in the patent. Therefore, it disclosed the general concept of using

lower-conductivity wash solutions. Document D9 disclosed the use of 10 mM Tris at pH 7.0 as the wash solution. This had been determined to have a conductivity of 0.99 mS/cm.

Since claim 1 included embodiments which represented a worse solution than the prior art, the objective technical problem could be formulated as the provision of an alternative, in the broadest sense of the term.

Document D11 already stated that buffers could be varied and disclosed different types of buffer (see paragraph [0106]).
The concept of using low-conductivity buffers for Protein A column chromatography was known in the art (see document D9, 10 mM histidine buffer and 10 mM Tris buffer). No technical effect could be recognised for a 8 mM Tris buffer as claimed versus a 10 mM Tris buffer as known from document D9.

For claims 8 and 9, the link between the wash solution and the effect was not part of the claims.

XVI. The requests of the parties were as follows.

The patent proprietors (appellant I) requested that the decision under appeal be set aside and the patent be maintained on the basis of the main request, submitted as auxiliary request 6 with the statement of grounds of appeal, or on the basis of auxiliary request 1, submitted as auxiliary request 7 with the statement of grounds of appeal. It also requested that document D53 be not admitted into the appeal proceedings.

Opponent 1 (appellant II) requested that the decision under appeal be set aside and the patent be revoked in its entirety and that document D53 be admitted into the appeal proceedings.

Opponent 3 (respondent) requested that appellant I's appeal be dismissed and the patent not be maintained on the basis of the main request.

Reasons for the Decision

Admittance of document D53 into the appeal proceedings

1. This document was filed by appellant II with its statement of grounds of appeal. It was cited in relation to inventive-step objections against claim 1 of the request held allowable by the opposition division, which is auxiliary request 1 in the appeal proceedings. Claim 1 is directed to the embodiment that uses deionised water.

2. The filing of document D53 in appeal proceedings constitutes an amendment of appellant II's case. Pursuant to Article 12(4) RPBA, it may be admitted into the appeal proceedings only at the discretion of the board.

3. In the current case, the feature "deionized water" was not included in the claims as granted. According to appellant II, the introduction of this feature from a single passage in the patent, i.e. paragraph [0051], could not have been expected, not least because the passage indicates that "[f]*or some applications*

deionized water is not suitable to be used in a wash step". However, the board considers that this is not determinant for the admittance of the document since the feature in question was already present in an auxiliary request filed during opposition proceedings, namely, in the sole embodiment of auxiliary request 10 filed on the final date set under Rule 116 EPC, which was two months before the oral proceedings.

4. The board notes that in opposition proceedings opponent 1 did not request that auxiliary request 10 not be admitted, nor did it request additional time to react. The argument that the patent as a whole did not envisage deionised water as a relevant embodiment is not seen as persuasive because one out of the seven examples with experimental results is dedicated to this embodiment.

5. In exercising its discretion, the board also considered the complexity of document D53, in accordance with Article 12(4) RPBA, and concluded that a detailed analysis of the document was required to establish the context in which deionised water was mentioned. The passage cited for disclosing deionised water was appendix 10.1. However, simply confirming that deionised water was mentioned in an appendix within a list of 96 buffers was not sufficient. Rather, it was necessary to establish whether deionised water was listed as a suitable wash solution for chromatography. However, this information was not apparent from the list in the cited appendix 10.1. Additionally, the experiments described in the main body of the document include those in which the antibody to be purified is present, as well as those in which it is not. It was unclear from the appendix which of those experiments the listed buffers were used for. Furthermore, when

arguing that the classification of "Mili Q water" (deionised water) in appendix 10.1 as "(0)" did not mean that it did not remove some HCP from the chromatographic column, appellant II referred to passages on pages 4, 24, 34, 10 and 35. The board notes that these passages do not refer to a single experiment, nor does the document make a link between them. Moreover, as argued by appellant I, the context in which deionised water is used is not irrelevant since, according to several documents cited in the proceedings, challenges posed by removal of HCP from antibody solutions relate to the binding of HCP to antibodies, rather than to the chromatography column (see for example D27, page 158, left-hand column, second paragraph, first sentence and description of reference 37 on page 159, right-hand column, and D16, abstract). In view of appellant I's argument that in document D53 deionised water was not used in the separation of HCP from antibody, this is a further aspect for the board to consider when assessing the relevance of this document.

6. In light of the above, the board decided that document D53 was not to be admitted into the appeal proceedings (Article 12(4) RPBA).

Main request

Inventive step (Article 56 EPC)

7. The objections relied on documents D7, D8, D9, D11 and D53 as representing the closest prior art.

8. However, document D53 was not admitted into the appeal proceedings (see points 1. to 6. above). Therefore, it will not be considered further.

Closest prior art
Document D11

9. Document D11 was taken by the opposition division and the patent proprietor to represent the closest prior art because it addressed the removal of PLBL2. Documents D7, D8 and D9 also addressed the removal of HCP but did not mention specifically PLBL2.

10. Document D11, like the patent at issue, concerns purification of antibodies produced by CHO cell culture and their separation from PLBL2. It discloses several purification processes which include Protein A chromatography as one of the purification steps. To address the undesirable levels of PLBL2, one approach tested involved adding further washes in the Protein A chromatography step. As a result of testing various wash solutions, it was concluded that none was effective in reducing PLBL2 and that different approaches should be tried (see page 70, last paragraph). The preferred approach was a three-column purification process involving Protein A chromatography, followed by anion exchange chromatography and hydrophobic interaction chromatography (see page 74, last paragraph). The conditions for the Protein A chromatography step involved equilibration with 25 mM NaCl and 25 mM Tris at pH 7.7, loading, washing with equilibration buffer, followed by washing with high-salt buffer at pH 7.0 and finally eluting at pH 2.8 (see page 75, first paragraph). Improved removal was attributed to the hydrophobic interaction chromatography step (see page 76, last paragraph).

11. The use defined in claim 1 differs in the low-conductivity wash solution, defined as less than 0.5 mS/cm, instead of the high-salt wash solution. Three low-conductivity wash solutions are specified in claim 1 in items (i), (ii) and (iii).

Technical effect and objective technical problem

12. The patent includes experimental results for PLBL2 removal obtained with several wash solutions according to the patent and several high-conductivity wash solutions according to the prior art. For the following reasons, a comparison of these results reveals that no improvement can be attributed to low-conductivity wash solutions in general.

12.1 According to Appellant I, the patent tested a range of low-conductivity solutions, from deionised water to 6 mM Tris at pH 8.0, each resulting in improved removal of PLBL2 relative to the control or to the load solution.

12.2 However, the board notes that the comparison to the control in the experiments in the patent is not relevant for identifying the technical effect of a low-conductivity solution (as claimed) versus a high-conductivity solution (as in document D11). Rather, referring to Example 2, the comparison between experiment b or c (wash with a low-conductivity buffer) and experiment d or f (wash with a high-conductivity buffer) is relevant. The table in Example 2 indicates better removal of PLBL2 with the experiments using low-conductivity solutions. However, since they further include an additional wash step (with an equilibration buffer), it is not possible to attribute the difference to the low-conductivity wash alone. The same design of

the experiments is seen in Examples 3 to 5. Moreover, even with the additional wash step, Examples 3 to 5 do not show improved PLBL2 reduction with the process using a low-conductivity solution. To the contrary, in all these examples (i.e. Examples 3 to 5), there is a greater reduction in experiment c (high-conductivity wash) compared to b (low-conductivity wash). Example 6 does not allow the relevant comparison because all experiments use a high-conductivity wash. Apart from Example 7, the patent contains no examples where the difference to the control lies merely in the low-conductivity wash.

12.3 As regards Examples 3 to 5, appellant I also relied on the yield rather than an increased PLBL2 reduction. According to this argument, the technical effect achieved by the low-conductivity wash would be a lower purity and a higher yield. If this were to be taken into account to formulate the objective technical problem, the problem would also be the provision of an alternative use. At oral proceedings, this argument was not further developed by appellant I.

12.4 Appellant I argued that Example 7 demonstrates that deionised water achieved superior results when compared to high-salt solutions - reference to Example 7Ae versus a, c and d; 7Ce versus a, c and d; and 7Ed versus a. The board agrees that indeed the results in Example 7 show that deionised water results in improved removal of PLBL2.

12.5 However, in view of the experimental results as a whole, as set out above, it does not seem possible to conclude that the low conductivity of the solution is on its own responsible for the increased removal since in some examples there was no removal that could be

attributed to this wash step. Removal was only consistently observed in Example 7, i.e. only with deionised water.

13. In view of the distinguishing feature and the technical effects that may be attributed to it, the objective technical problem is the provision of an alternative wash solution for use in a wash step in Protein A chromatography for reducing PLBL2 in the purification of an IgG4 or IgG1 antibody.

Obviousness

14. The skilled person seeking to provide an alternative wash solution would use any known wash solution. The choice of a particular one among the known wash solutions does not involve an inventive step. Tris and phosphate buffers were known wash solutions (see for example document D9, disclosing the wash solution 10 mM Tris at pH 7.0). The concentration range indicated in claim 1, i.e. "about 0.1 mM to about 8 mM", is not associated with any technical effect, as set out above (see points 12. to 12.5). The argument that a higher concentration would result in increased conductivity is not persuasive as both lower and higher conductivities were among the options the skilled person would consider when seeking to provide an alternative.

15. In conclusion, no inventive step can be recognised for the use of a wash solution with the conductivity defined in claim 1 given that no technical effect over the prior art could be attributed to the low conductivity. No technical effect could be attributed to the characteristics of the Tris buffer in item (i) or the phosphate buffer in item (ii) either. Tris and phosphate buffers are considered to be wash solutions

that the skilled person had at their disposal when seeking to solve the problem as posed. Therefore, the subject-matter of claim 1 does not involve an inventive step (Article 56 EPC).

In view of this finding, there was no need to address the objections starting from the other documents identified as closest prior art.

Auxiliary request 1

Inventive step (Article 56 EPC)

Closest prior art as represented by document D9

16. Document D9 addresses purification methods involving chromatography for the removal of HCP in general. Its focus lies in the addition of an amino acid to the buffer solutions used at any stage of the chromatography steps such as Protein A chromatography. Two embodiments were highlighted by appellant II: 10 mM Tris at pH 7.0 and 10 mM histidine at pH 7.0 (Examples 2 and 4 in document D9). The latter has a conductivity of 0.1 mS/cm, according to calculations submitted by opponent 4 during opposition proceedings (see decision under appeal, point 6.3.4).

17. Claim 1 is directed to the embodiment using deionised water. This is a distinguishing feature over document D9. As regards the feature PLBL2, appellant II contested that it constituted a further difference to document D9. This feature is not considered in the assessment below.

Technical effect and objective technical problem

18. Appellant II argued that deionised water resulted in poorer removal than the wash solution in document D9. This conclusion could be drawn from Example 7E of the patent because the removal was worse for deionised water than for wash solutions b and c.

19. However, a comparison with the wash solutions of document D9 is not possible from Example 7E in the patent. The wash solutions b and c of Example 7E in the patent are 31 mM Tris at pH 8.5 and 55 mM Tris at pH 9.0, respectively, which could be considered to have "intermediate conductivity". Document D9 does not disclose any level of removal achieved with such wash solutions. Therefore, Example 7E of the patent does not provide a direct comparison between deionised water and the prior art disclosed in document D9.

20. In support of its argument that deionised water resulted in poorer removal, appellant II further referred to wash solutions b and c in Example 7E that achieved superior removal than deionised water. However, the comparison between deionised water and other wash solutions in the patent is not relevant for assessing the technical effect over the prior art.

21. Thus, no comparison can be made with the use disclosed in the closest prior art that would show a worse removal. The only technical effect that can be attributed to the use of deionised water is PLBL2 removal (as set out in point 12.4 above).

22. In view of the technical effect, the objective technical problem may be formulated as the provision of an alternative wash solution for use in a wash step in

Protein A chromatography for reducing PLBL2 in the purification of an IgG4 or IgG1 antibody.

Obviousness

23. None of the cited documents discloses deionised water as a wash solution in chromatography. The argument that any low-conductivity wash solution is an obvious solution is not convincing given that not all low-conductivity wash solutions achieve removal of PLBL2, as set out above for the main request (see point 12.5 above). In view of this finding, the arguments relating to the skilled person's knowledge about the hydrophobic reversible interactions between PLBL2 and antibodies and about conductivity are moot. Moreover, while documents D7 to D9 disclose purification methods involving wash steps of controlled conductivity, none discloses that the relevant feature of the wash solution is a conductivity of 0.5 mS/cm or less.

24. Objections were also raised starting from document D26. The contents of this document, relevant to the use defined in claim 1, do not go beyond those in document D9. Therefore, the reasoning set out above applies *mutatis mutandis* to those objections.

Closest prior art as represented by document D7, D8 or D11

25. The reasoning above for document D9 also applies when starting from the disclosure in document D7, D8 or D11.

25.1 Document D7, like the patent, concerns the removal of HCP in the purification of antibodies. Unlike the patent, it does not address the HCP PLBL2. It discloses a process involving several chromatography and filtration steps. The chromatography step involves

multiple wash steps, differing in the conductivity or pH of the wash buffer. In some embodiments, a wash step with a solution of conductivity in the range of 1 to 5 mS/cm is followed or preceded by a wash solution of a conductivity above 20 mS/cm (see page 3, second to third paragraphs, page 6, second to third paragraphs and paragraph bridging pages 10 to 11).

25.2 Document D8 does not disclose any additional features compared to D7.

25.3 The contents of document D11 were summarised above in point 10. Although this document specifically addresses the HCP PLBL2, which is not mentioned in document D9, the reasoning for document D9 applies here too as the arguments relating to the skilled person's knowledge of the hydrophobic reversible interactions between PLBL2 and antibodies and of conductivity were also taken into consideration.

26. In conclusion, none of the arguments that the subject-matter of claim 1 is obvious were persuasive. Claims 7 and 8 are directed to a method of producing and a method of purifying a human IgG4 or IgG1 isotype antibody, respectively, and include a step of washing the Protein A chromatography material with deionised water. Therefore, this subject-matter is not obvious for the same reasons as claim 1.

Amendments (Article 123(2) EPC)

27. The feature "deionized water" has a literal basis in the application as filed, in the passage on page 9, line 36 to page 10, line 3, which reads: "*In one embodiment of all aspects the low conductivity aqueous solution is deionized water. For some applications*

deionized water is not suitable to be used in a wash step. In some embodiments the low conductivity aqueous solution is not deionized water."

28. According to a first argument, the amendments are not allowable as this passage in fact disclaims the feature in question. The board does not agree with this reading and considers that the first sentence in this passage discloses deionised water as generally applicable to all embodiments of the invention. The second sentence disclaims deionised water for "*some applications*". However, in the board's view, it neither disclaims its use in general nor specifies the "*some applications*" for which it is not suitable. Thus, the disclaimer is not generally applicable but limited to unspecified applications. Its combination with specific applications could go beyond the content of the application as filed. The converse is, however, not true, i.e. the fact that there is no basis for combining the disclaimer with specific applications does not imply that there is no basis for combining the positive feature, i.e. deionised water, with every "*application*" in the application as filed.

29. According to a second argument, the combination of this feature with the feature PLBL2 extended beyond the content of the application as filed since deionised water had not been disclosed in combination with PLBL2. However, as set out in point 28., deionised water was explicitly disclosed as being generally applicable to all aspects of the invention, and therefore it was equally applicable to the two specifically mentioned HCPs, clusterin and PLBL2.

30. In summary, the combination of features in claim 1 is directly and unambiguously derivable from claims 1

and 2 in combination with the above-discussed passage of the application as filed (page 9, line 36 to page 10, line 3).

Disclosure of the invention (Article 83 EPC)

31. According to one argument, all the examples use the same setting, in which the low-conductivity wash step is preceded by a high-conductivity wash step and an intermediate-conductivity wash step and is immediately followed by an elution step with a higher-conductivity solution. Since this setting is not reflected in features in claim 1, the claimed invention lacks disclosure. A reduction in PLBL2 content with a low-conductivity solution as the sole wash solution is not sufficiently disclosed. Documents D7 and D14 illustrated the required wash steps in Protein A chromatography.

32. The board does not find this argument persuasive.

32.1 Firstly, the claim does not specify a level of reduction in PLBL2. Therefore, it suffices that any reduction in PLBL2 content is achieved, there being no requirement that the reduction be greater than with any other wash solution.

32.2 Secondly, as regards the sequence of wash steps used in the experiments, the board notes that only Example 7 is relevant for the invention defined in claim 1. Therefore, it is not necessary here to address the arguments based on Examples 2 to 6.

32.3 Appellant II argued that this wash step is preceded and followed by washing with a high-conductivity solution. The board considers that washing the column for three

column volumes with the equilibration buffer after loading (see paragraph [0139] of the patent) was a routine step. Since all the wash solutions tested in Example 7 used this same routine step and nevertheless deionised water resulted in superior PLBL2 removal compared to most other wash solutions, it does not demonstrate that the use of deionised water to reduce the PLBL2 content cannot be carried out. In other words, the wash with deionised water results in removal of PLBL2. Appellant II has not provided any data showing the contrary. Therefore, the effect in the claim is demonstrated in the patent and patent application.

32.4 The fact that documents D7 and D14 describe a given sequence of wash steps involving higher- and lower-conductivity solutions does not demonstrate that the use of a low-conductivity wash step only will not achieve a reduction in the HCP PLBL2. It is necessary to distinguish between the effect defined in claim 1, namely a reduction in PLBL2 content, and a question of improved removal, which is not a requirement of claim 1.

32.5 Claims 2 and 9 define an additional wash step with a high-conductivity and/or medium-conductivity aqueous solution which, in one embodiment, follows the low-conductivity wash step. Also for the invention defined in these claims, the considerations in points 32.1 to 32.4 apply.

33. According to a second argument, the statement in paragraph [0051] of the patent that deionised water is not suitable for some applications implied that claim 1 included non-working embodiments. However, the application contained no teaching as to the

circumstances under which deionised water was suitable or unsuitable.

34. The board considers that the statement in question does not on its own suffice to conclude that the invention defined in claim 1 is not sufficiently disclosed as regards a wash solution consisting of deionised water.

34.1 The patent shows the purification of six different antibodies with Protein A chromatography (Examples 7A to 7E). All examples using deionised water as the wash solution achieved a reduction in PLBL2 content. In view of these experimental results, the statement in paragraph [0051] of the patent is not supported by any example. While that statement is not followed by guidance on how to avoid failures, appellant II has not submitted any experimental evidence to substantiate doubts in this respect either.

35. According to yet another argument, the separation of PLBL2 from an antibody solution depends on the antibody in question. Thus, the invention defined in claim 1 is not sufficiently disclosed for antibodies other than those in the examples.

36. This argument is not convincing. A preferential interaction of PLBL2 with IgG4 as compared to IgG1 (disclosed in document D13) is not enough to call into question the reproducibility of the claimed use as concerns IgG4. The behaviour of different IgG subtypes and individual antibodies relative to PLBL2 does not on its own demonstrate that a low-conductivity solution is not suitable for disrupting the association between the two as such a solution is not mentioned in the document. The same applies to the argument that the

separation depends on the CDRs of the specific antibody.
Documents D5 and D6 are also cited for their disclosure of the nature of the interaction between PLBL2 and antibodies. Therefore, nor do these contain a disclosure regarding the effect of a low-conductivity solution.

37. In conclusion, the arguments contesting sufficiency of disclosure for the inventions defined in claims 1, 2 and 9 were not convincing.

Order

For these reasons it is decided that:

The appeals are dismissed.

The Registrar: The Chairwoman:

C. Vodz M. Pregetter

Decision electronically authenticated