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D E C I S I O N
of 30 September 2004

Case Number: T 1090/02 - 3.3.4

Application Number: 91919421.7

Publication Number: 0553234

IPC: C12Q 1/66

Language of the proceedings: EN

Title of invention:
Luciferase Compositions and methods

Patentee:
Promega Corporation

Opponents:
01) Roche Diagnostics GmbH
02) Winfried Scheirer

Headword:
Luciferase/PROMEGA

Relevant legal provisions:
EPC Art. 54, 56, 83, 123(2)

Keyword:
"Added subject-matter (no)"
"Sufficiency of disclosure (yes)"
"Novelty (yes)"
"Inventive step (yes)"

Decisions cited:
G 0002/88, T 0279/93, T 0892/94, T 0706/95

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1090/02 - 3.3.4

D E C I S I O N
of the Technical Board of Appeal 3.3.4
of 30 September 2004

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-

Decision under appeal:
Interlocutory decision of the Opposition Division of the European Patent Office posted 16 July 2002 concerning maintenance of European patent No. 0553234 in amended form.

Composition of the Board:

Chairman: S. C. Perryman
Members: M. Wieser
A. L. L. Marie

Summary of Facts and Submissions

I. The appeal was lodged by the Opponents 02 (Appellants) against the decision of the Opposition division, whereby the European patent No. 553 234, was maintained in amended form pursuant to Article 102(3) EPC.

II. The patent had been opposed by two parties (Opponents 01 and 02) under Article 100(a) on the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC), under Article 100(b) EPC on the ground of lack of sufficient disclosure (Article 83 EPC) and under Article 100(c) EPC on the ground of added subject matter (Article 123(2) EPC).

III. The Opposition Division had decided that claims 1 to 42 of the main request before them, submitted at oral proceedings on 7 March 2002, met the requirements of the EPC.

IV. Independent claims 1, 14, 27, 33 and 40 of this main request read:

"1. A method for detecting the presence of a beetle luciferase in a sample suspected of containing the luciferase, comprising:

a) admixing an aliquot of the sample with an aqueous solution comprising a thiol reagent other than CoA, luciferin, adenosine triphosphate, and Mg^{2+} all at concentrations effective for activity of the luciferase in the luciferase/luciferin reaction, and wherein the aqueous solution does not contain a luciferase, and

b) measuring the luminescence from the solution resulting from step a), wherein the solution resulting from step a) comprises thiol reagent other than CoA at a concentration above 5mM.

14. Use of a thiol reagent other than CoA at a concentration between 5mM and 200mM to extend the kinetics of light production in a beetle luciferase/luciferin reaction in a method for the detection of ATP in a sample suspected of containing ATP, comprising

a) admixing an aliquot of the sample with an aqueous solution comprising the said thiol reagent other than CoA, a beetle luciferase, luciferin and Mg^{2+} all at concentrations effective for activity of the luciferase in the luciferase/luciferin reactions, and

b) measuring luminescence from the solution resulting from step a).

27. An aqueous solution comprising a beetle luciferase, which is capable of catalyzing the luciferase-luciferin reaction, a thiol reagent other than CoA at a concentration of between 5mM and 200mM, and CoA at a concentration between 0.1mM and 1.0mM.

33. A test kit for assaying for the presence of a beetle luciferase in a sample thought to contain such a luciferase, said kit comprising a luciferase-luciferin reaction composition which is an aqueous solution comprising a thiol reagent other than CoA at a

concentration of between 5mM and 200mM, adenosine triphosphate, luciferin, and Mg^{2+}, wherein the reaction composition is free of a luciferase.

40. A test kit for assaying for the presence of adenosine triphosphate in a sample thought to contain said compound, said kit comprising an aqueous solution comprising CoA at between 0.1mM and 1.0mM, a beetle luciferase, a thiol reagent other than CoA at a concentration between 30mM and 80mM, luciferin at between 0.1mM and 1.0mM, and magnesium ion at between 2mM and 15mM."

These claims are the same as the claims as granted except for the addition of the passage reading "wherein the solution resulting from step a) comprises thiol reagent other than CoA at a concentration above 5mM" at the end of claim 1.

V. The Board expressed their preliminary opinion in a communication dated 7 May 2004.

Oral proceedings were held on 30 September 2004. Opponents 01, being a party as of right according to Article 107 EPC, though duly summoned, were not represented.

VI. The Appellants requested that the decision under appeal be set aside and that the patent be revoked.

The Patent Proprietors (Respondents) requested that the appeal be dismissed.

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

(2) Biochem. Biophys. Acta, vol.27, 1958, pages 519-532

(4) US-4 246 340

(6) EP-0 015 437

(7) Anal. Biochem., vol. 52, 1973, pages 449-455

(8) J. Appl. Biochem., vol. 2, 1980, pages 469-479

(11) Anal. Biochem., vol. 171, 1988, pages 404-408

(12) US-4 235 961

(19) Clin. Chemistry, vol. 18, No. 5, 1972, pages 473-475

(20) Annex A, submitted by the Appellants on 26 November 2002

VIII. The submissions made by the Appellants may be summarized as follows:

All independent claims contravened the requirements of Article 123(2) EPC. The same applied to dependent claims 7, 8, 20, 21, 34, 35 and 41. All these were claims which specified certain components and their concentrations in combinations not disclosed in the application as originally filed, because in the application as filed the relevant concentrations were

only specified for solutions also containing other components which the claims objected to did not require. This amounted to the addition of subject matter and made these claims unallowable.

In the light of the case law of the Boards of Appeal, mention in claim 14 of "use ... to extend the kinetics of light production "could not be treated as a technical feature which distinguished the claim from the disclosures in documents (6), (7) and (12). Consequently, the subject-matter of claim 14, and of claims 15 and 24 which depended thereon was not new, contrary to the requirement of Article 54 EPC. Document (6) moreover anticipated the subject-matter of claims 25 and 26.

Claims 27, 31, 40 and 42 lacked novelty over the disclosure in document (2).

The subject-matter of claim 32, referring to a composition containing a beetle luciferase, ATP and luciferin was unable to solve a technical problem and therefore lacked an inventive step (Article 56 EPC).

Moreover, no technical problem was solved by the use of a thiol reagent other than CoA at a concentration of at least up to 10mM and by the use of DTT at concentrations higher than 200mM.

All independent claims lacked an inventive step over the cited prior art documents. A skilled person starting from any of documents (2), (4), (6), (8) or (12) would arrive at the claimed subject-matter in an obvious way.

Dithiothreitol (DTT) was the only thiol reagent other than Coenzyme A (CoA) referred to in an example of the patent. Bovine Serum Albumin (BSA) was the only proteinaceous luciferase-activity enhancer referred to in an example of the patent. The determination of what other thiol reagents or luciferase-activity enhancers would work amounted to undue burden for the skilled person, contrary to the requirements of Article 83 EPC.

IX. The submissions made by the Respondents may be summarized as follows:

The utility in practice of the luciferin/luciferase reaction was limited due to the brevity and pattern of the light emission. The patent was concerned with two mechanisms caused by two different substances, CoA and a thiol reagent other than CoA, which independently, and in different ways contributed to an improvement of the kinetics of the light production of this enzymatic reaction. All specific concentrations claimed for these substances were disclosed in the application as originally filed. As the skilled reader would appreciate that the effect caused by CoA was totally independent of the effect caused by a thiol reagent other than CoA, and vice versa, the skilled reader would understand that for the mentioned concentrations of a thiol reagent other than CoA the desired effect would be achieved whether or not CoA was present. The omission of a reference to CoA in the claims did thus not add subject matter, and the claims met the requirements of Article 123(2) EPC.

The extension of the kinetics of light production in a beetle luciferin/luciferase reaction by the addition of a thiol reagent other than CoA represented a technical effect, which according to the established case law of the Boards of Appeal was a technical feature of claim 14. None of the documents cited by the Appellants mentioned this effect: it could thus be treated as a hidden technical effect in the sense of the case law. The subject-matter of the claims was therefore novel under Article 54 EPC.

All embodiments falling within the scope of the claims solved the technical problem underlying the invention, to improve the kinetics of light production for the enzymatic reaction, and therefore were based on an inventive concept (Article 56 EPC).

None of the documents cited by the Appellants was a proper starting point for assessment of inventive step following the problem and solution approach. Documents (2), (6), (8) and (12) did not identify the same problem to be solved as in the patent in suit, nor had they considered using thiol reagents other than CoA for any purpose other than their well known enzyme stabilizing effect.

A person skilled in the art was given clear and precise information in the patent how to select and identify suitable thiol reagents and luciferase-activity enhancers in addition to those explicitly mentioned in the examples and the description. The Appellants, merely alleged that the invention was not sufficiently disclosed over the whole ambit of the claims

(Article 83 EPC), but did not provide any evidence substantiating this allegation.

X. The Opponents 01 did not make any submissions during the appeal procedure.

Reasons for the decision

Added subject matter - Article 123(2) EPC

1. The feature in claim 1 that is alleged to add subject matter is the requirement at the end of the claim that the solution resulting from admixing an aliquot of the sample to be tested as to whether it contains beetle luciferase comprises a thiol reagent other than CoA at a concentration above 5mM. While Claim 1 as originally filed, and the original description disclosed the use of thiol reagents even in the absence of CoA, there is no explicit statement of the concentration for such reagent in these circumstances either in the claims or the description.

2. Claim 14 is alleged not to have a basis in the application as originally filed because it requires a thiol reagent other than CoA at a concentration between 5mM and 200mM without also requiring the presence of CoA. There is no explicit disclosure in the original application of the range of concentration of the thiol reagent other than CoA which can be used in the absence of CoA.

3. The application as filed described that CoA interacts with luciferase and electronically excited oxyluciferin

during catalysis of the luciferase-luciferin reaction and, as one consequence of this interaction, reduces product inhibition of the enzyme in the course of the reaction (page 21, lines 27 to 32 of the original application). Figure 1 shows that the addition of CoA to the assay mixture, in the absence of a thiol reagent other than CoA, yields a greater initial light intensity with a lower initial decay rate, and more than a two-fold increase in total luminescence. This effect of CoA on the kinetics of beetle luciferase - luciferin reaction saturates at relatively low CoA concentrations, between 0.1mM and about 1.0mM, which is a range typical for saturation of binding to an enzyme by a substrate (page 23, line 34 to page 24, line 2).

4. From the passage at page 24, lines 3 to 19 of the application as originally filed the skilled reader learns that such thiol reagent at a concentration above 5mM has a stabilizing effect on the enzyme while the catalysis proceeds, and that this effect cannot be attributed simply to protection generally against oxidation of groups on the enzyme. (This general protection against oxidation was known in the art as evidenced by documents (4), (7), (8) and (19)). This stabilizing effect during catalysis, which on page 24, lines 5-6 of the original application is said to be saturated at between about 30mM and about 80mM, is shown in figure 2, in the absence of CoA. The original peak height is reduced, the curve shows a much higher "steady -state" over a prolonged period of time and total light production is increased over that without DTT.

5. On page 9, lines 8-12 of the original application it is said that including CoA, or a thiol reagent such as DTT, or both, in a beetle luciferase-luciferin reaction mixture provides surprising improvements in the kinetics of light production from the reaction and in the total yield of light from the reaction. While the specific examples also use CoA as well as a thiol reagent other than CoA, there is no suggestion that the concentrations of these two types of components are to be adjusted in a mutually dependent way, and the skilled reader must assume that the concentrations of the components can be chosen independently of one another. The concentrations for thiol reagent other than CoA range from 5mM to 200mM. The skilled reader can derive the clear and unambiguous information that for the method of claim 1 the concentration of thiol reagent other than CoA should be above 5mM. That the reader is also told that the preferred or optimal concentrations are higher than this is not relevant for the purposes of Article 123(2) EPC.

6. In the light of figures 1 and 2 and the passages of the description cited above, the Board comes to the conclusion that the effects of CoA and of thiol reagents other than CoA on the kinetics of light production in a beetle luciferase-luciferin reaction are independent from each other. These effects are moreover not dependent on the influence resulting from the addition of proteinaceous luciferase-activity enhancers, like BSE, as described for instance in prior art documents (4) and (8).

7. In this situation the Board is of the opinion that claims to the use of these compounds, or to solutions

and kits containing them, in different, specific concentrations do not need to have a literal basis in a single passage of the application as originally filed, as long as the exact concentrations and ranges claimed for the specific substances are disclosed as such in the original application. The reference in a claim to a combination of these compounds in specific concentrations, explicitly disclosed in different passages of the original application, is not considered to be an amendment of the patent which extends beyond the content of the application as originally filed.

8. The lower limit for a thiol reagent other than CoA according to claim 1, namely above 5mM, is disclosed on page 24, line 13.

9. The concentration for CoA, lying between 0.1mM and 1.0mM, according to claims 7, 8, 20, 21, 27 and 40 is disclosed at various positions in the original application (for instance in the sentence bridging pages 23 and 24). The concentration for BSA indicated in claims 8 and 21, namely between 10µg/ml and 5mg/ml, has a basis on page 25, line 4.

10. The concentration range for a thiol reagent other than CoA according to claims 14, 27 and 33, lying between 5mM and 200mM, is disclosed in original claims 24 and 41.

11. Thiol reagents other than CoA in a concentration between 30mM and 80mM, according to claims 34 and 40, are disclosed on page 24, line 6 as filed. ATP at between 0.1mM and 1.0mM referred to in claim 34 is disclosed on page 24, line 29. Luciferin at between

0.1mM and 1.0mM and magnesium ion at between 2mM and 15mM, according to claims 34 and 40 have a basis on page 24, lines 29 to 31 as originally filed.

12. Consequently the Board decides that claims 1 to 42 meet the requirements of Article 123(2) EPC.

Sufficiency of disclosure - Article 83 EPC

13. The Appellant has objected that only DTT is exemplified as a thiol reagent other than CoA, and that the determination of what other thiol reagents would work would amount to undue burden.

14. Page 7, lines 1 to 5 of the patent defines the term "thiol reagent other than CoA" by structure (it has a free sulfhydryl group) and by function of this SH-group (that it is effective as a reducing agent at the conditions at which the luciferin-luciferase reaction is carried out). DTT is indicated to be the preferred reagent, and a list of five other candidate substances is given. The Appellant has not shown that any of these would not work. The Board does not consider that any case of insufficiency has been made out in this respect of the feature of the claim "thiol agent other than CoA".

15. The Appellant has also objected that the additional feature of "luciferase-activity enhancer" of claim 4 is not sufficiently defined to meet the requirements of Article 83 EPC, and as the determination of all reagents which would work would amount to undue burden.

16. Page 9, lines 45 to 52 of the patent discloses that compositions according to the invention may also comprise a proteinaceous luciferase-activity enhancer such as mammalian serum albumin or lactalbumin or an ovalbumin, preferably BSA. BSA is the only one used in the examples. The Appellant has not shown that any of the others would not work.

17. In the light of this disclosure in the patent in suit the Board does not see that the identification of proteinaceous luciferase-activity enhancers, other than BSA, amounts to undue burden for the skilled person for the purposes of Article 83 EPC.

Novelty - Article 54 EPC

18. Novelty of claim 1 relating to a method for detecting beetle luciferase has not been challenged, but the novelty of claim 14 relating to a "Use of a thiol reagent other than CoA at a concentration between 5mM and 200mM to extend the kinetics of light production in a beetle luciferase/luciferin reaction in a method for the detection of ATP in a sample suspected of containing ATP..." has been challenged on the basis that this use could not be treated as a technical feature, and that therefore the claim was not novel over documents (6), (7) and (12).

19. The use to which claim 14 is directed is different to the known prior art use, as it relates to a modification of the reaction catalysed by the enzyme, and not the known stabilizing function against accidental oxidation. For a document to anticipate this use the document should disclose that the thiol reagent

affects the kinetics of the reaction which luciferase catalyses.

20. Document (6) refers to a process and a reagent for determination of Creatinkinase (CK). The CK-activity is determined by measuring the formation of ATP using the luciferin-luciferase reaction (page 2, equations (1) and (5)). According to page 3, lines 31 to 34, it is preferable to add to the reaction mixture an organic sulfohydryl compound such as N-acetylcystein (NAC), DTT, dithioerythritol and reduced glutathione, at concentrations between 1 and 100mM, preferably between 2 and 50mM (page 4, lines 15 to 16 and 22). Solution 2 used in example 1 contains 33mM/l NAC, which results in a NAC concentration in the test of 10mM/l (page 5, line 25).

21. Whereas it neither indicates the purpose for the addition of the sulfohydryl compound, nor any effect caused thereby, document (6) does, however, on page 3, lines 57 to 62, indicate that AMP, added to inhibit adenylate kinases (also designated "myokinases", see page 2, lines 25 to 30), modifies the properties of luciferase, so that the product inhibition by oxyluciferin is eliminated. Thus, when a defined ATP concentration is measured, instead of a flash-like signal-time curve a substantial constancy of signal over more than 15 minutes is achieved. The effect achieved by the addition of the luciferase inhibitor AMP is depicted in figure 1 of document (6). Thus document (6) describes a substance AMP added to modify the luciferase kinetics. The reader can only assume that the sulfohydryl compound is not present for the

same purpose, and accordingly document (6) does not destroy the novelty of claim 14.

22. Document (7) discloses an automated method for ATP analysis utilizing the luciferin-luciferase reaction. It is mentioned in the abstract that the firefly extract is stabilized with BSA and mercaptoethanol, which substances are contained in a dilution buffer. Without BSE and mercaptoethanol, the luciferase containing extract is said to begin to lose its activity after one hour (page 454, end of first paragraph). According to page 451 (bottom of page) the used luciferase extract contains $NaAsO_4$, a luciferase inhibitor. There is no suggestion that the mercaptoethanol is used to modify the luciferase kinetics, and document (7) cannot be regarded as destroying the novelty of claim 14.

23. Document (12) describes a method for photometric determination of the subunit B of CK. Two subunits of the enzyme exist in human tissue, namely CK-M and CK-B. The CK-B activity is determined by measuring the formation of ATP using the luciferin-luciferase reaction in the presence of a thiol protecting reagent, such as NAC or DTT, in a preferred concentration of 2 to 50mM (document (12), column 3, lines 15 to 19). While no specific effect is mentioned to result from the addition of a thiol protecting reagent, it is said in column 2, lines 21 to 27, that the addition of an antibody inhibiting CK-M and of L-Luciferin, a competitive luciferase inhibitor, made it possible to determine CK-B and to simultaneously obtain a continuous light emission proportional to the enzyme activity of CK-B which makes a kinetic monitoring of

the light emission possible. Document (19) does not tell the reader to use the thiol protecting reagent to modify the kinetics of the luciferase reaction, rather something else is added for this purpose. Document (12) does not anticipate the use claimed in claim 14.

24. Document (19) describes that the highest activation of CK can be achieved by adding a thiol compound at a concentration of 10mM/l. This activation is a thermodynamically-controlled not a kinetically-controlled phenomenon (see document (19) abstract and page 474, left column, third full paragraph). The Appellants argument, based on document (19), that the amount of thiol reagents added to the reaction mixtures in documents (6), (7) and (12) by far exceeds the amount which is described in the prior art as having a beneficial effect on the stability and activity of enzymes, and thus must be for the purpose of modifying the kinetics, thus cannot be accepted by the Board.

25. Article 54(2) EPC defines the state of the art as comprising "everything made available to the public by means of a written or oral description, by use or in any other way". The word "available" carries with it the idea that, for lack of novelty to be found, all the technical features of a claim in combination must have been communicated to the public. A line must be drawn between what has in fact been made available and what remains hidden. Moreover, it is of no relevance what may have been inherent in what is made available by the prior art as the question of inherency does not arise as such under Article 54 EPC (cf. G 2/88, supra, point (10.1) of the reasons for the decision).

26. None of document (6), (7) and (12) teach use of thiol reagents other than CoA in a beetle luciferase/luciferin reaction for the detection of ATP to extend the kinetics of light production.

27. The Appellants argue that no novelty exists if a claim is directed to the use of a **known substance**, for a **known purpose**, even if a **newly discovered technical effect** underlying said known use is indicated in the claim. They refer in this respect to decisions T 279/93 of 12 December 1996, T 892/94 of 19 January 1999, T 706/95 of 22 May 2000, where the competent Boards were confronted with exactly this situation. But the Board considers the present situation different because the reader of documents (2), (7) and (12) is given no reason to assume that a thiol reagent is being added to extend the kinetics of light production, rather than for its known purpose of stabilizing enzymes.

28. For these reasons the subject-matter of claim 14 is novel and meets the requirements of Article 54 EPC. The same applies to dependent claims 15, 24, 25 and 26.

29. Claim 27 (and claim 31 dependent thereon) and claim 40 (and claim 42 dependent thereon) are alleged to lack novelty over document (2). Claim 27 refers to an aqueous solution comprising a beetle luciferase capable of catalyzing the luciferase-luciferin reaction. The solution contains a thiol reagent other than CoA and CoA in specifically indicated concentrations. Claim 40 relates to a test kit for assaying for the presence of ATP in a sample. The kit comprises an aqueous solution comprising CoA, a thiol reagent other than CoA,

luciferin and magnesium ion, all in specifically indicated concentrations, and a beetle luciferase.

30. Document (2) relates to the function of CoA in luminescence. The document describes the reaction of luciferin with ATP to reversibly form adenyl-luciferin and pyrophosphat. Adenyl-luciferin (active luciferin) reacts with molecular oxygen to give light emission and adenyl-oxyluciferin, which is a luciferase inhibitor. The addition of CoA to the reaction mixture stimulates luminescence, due to the removal of the inhibitor from the enzyme surface. Adenyl-oxyluciferin reacts with CoA to form oxyluciferyl-CoA. In the presence of cysteine (a thiol reagent other than CoA) the CoA derivative is converted into stable N-oxyluciferyl-cysteine.

31. Figure 6 on page 524 of document (2) shows the effect of cysteine on oxylucferyl-CoA fluorescence. 0.1mM cysteine is added to the reaction mixture, which is said to be the same as described for figure 5. Figure 5 shows the fluorescence change on alkaline hydrolysis of oxyluciferyl-CoA. The initial reaction mixture examined according to figure 5 is said to be the same as the one described in figure 4. Figure 4 shows the effect of CoA on oxyluciferin fluorescence. The conditions are described as being the same as for figure 2, except oxyluciferin replaced luciferin. The reaction mixture underlying the bioluminescence measurement shown in figure 2 contains luciferin, luciferase, ATP and CoA.

32. Accordingly, the reaction mixtures underlying the fluorescence measurements shown in figures 4, 5 and 6 do not contain luciferin, but instead the luciferase inhibitor oxyluciferin.

33. Thus, document (2) does not disclose an aqueous solution capable of catalyzing the luciferase-luciferin reaction comprising CoA and a thiol reagent other than CoA according to claim 27, or a test kit for assaying the presence of ATP comprising luciferin, CoA and a thiol reagent other than CoA according to claim 40.

34. Said claims and dependent claims 31 and 42 are therefore novel in the meaning of Article 54 EPC.

Inventive step - Article 56 EPC

Closest prior art and problem to be solved

35. In accordance with the problem and solution approach, the Boards of Appeal in their case law have developed certain criteria for identifying the closest prior art providing the best starting point for assessing inventive step. It has been repeatedly pointed out that this should be a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most relevant technical features in common, i. e. requiring the minimum of structural modifications (cf. Case Law of the Boards of Appeal of the European Patent Office, 4th Edition 2001, chapter I.D.3.1).

36. While the Appellants, during oral proceedings considered any of documents (2), (6) and (8), and during the written procedure additionally documents (4) and (12), as being an equally suitable potential starting points for the application of the problem and solution approach, the Board is of the opinion that

only document (8), referring to the optimization of firefly luciferase assay for ATP, represents a suitable starting point and thus the closest prior art for the subject-matter of all independent claims of the patent in suit. The other documents are more remote. Thus document (2) is concerned with the function of coenzyme A in luminescence, document (4) is specifically concerned with the use of one or more competitive inhibitors of luciferase such as D-luciferin analogues, document (6) relates specifically to a method of determining creatine kinase, and document (12) to a method for the photometric determination of the subunit of creatine kinase.

37. Document (8) discloses that BSA, EDTA and DTT stabilize luciferase and allow yielding more reproducible results (abstract). The activity of luciferase is determined in various buffer solutions containing 0.5mM/ml DTT (table I, page 470). According to table II on page 473, and page 475, first full paragraph, the DTT addition gives a 1.6-fold stimulation of light production of the luciferin-luciferase reaction, while mercaptoethanol and cysteine give a 1.1-fold stimulation. It has to be noted that the stimulation effected by BSA, according to table II, is 2.4-fold.

38. In the light of the disclosure in document (8) the problem to be solved by the patent in suit is seen as the provision of methods and means to enhance further the utility of beetle luciferase as reporter by improving the kinetics of light production for the enzymatic reaction. The aim is to effect more efficient light production, i. e. light emission at a more nearly

continuous, yet high rate (cf. page 3, lines 42 to 45 and 54 to 56).

39. This problem is plausibly solved by the method and the use according claims 1 and 14, wherein the aqueous solution and the test kits according to claims 27, 33 and 40 are employed.

40. In relation to certain claims the Appellants argue that these do not solve the above problem, and so cannot meet the requirement for an inventive step.

41. The composition of Claim 32, comprising luciferase, ATP and luciferin, is alleged by the appellants not to be useful, either for an luciferase or an ATP assay, as all components required for an immediate start of the reaction are already contained in it. The Respondents submitted however that the composition would be useful in an assay for the determination of a potential inhibitor of the enzymatic reaction. The Board agrees that this appears to be a use requiring the same problem to be solved as for the other claims.

42. By referring to page 10, lines 29 to 30 of the opposed patent, the Appellants argue that the technical problem is not solved with respect to a thiol reagent other than CoA at a concentration of at least 10mM. The passage referred to by the Appellants relates to preincubation of the enzyme with 10mM DTT which was found not to lead to an increased effect. This is different from the use of a the thiol reagent in the assay, i. e. during catalysis, which is the subject-matter of the claims and to which the problem to be solved relates.

43. Finally it was argued that the technical problem is not solved for DTT concentrations higher than 200mM. Such embodiments are covered by claim 1 which discloses a lower limit but no upper limit for the thiol reagent other than CoA. Appellants rely in this respect on experimental data provided in document (20).

44. However, the data submitted by the Appellants does not show that no technical effect is achieved at concentrations above 200mM. Rather the skilled person can learn from document (20) that the data show a maximum effect between 97 and 140mM, with a decline of the effect at higher concentrations. This cannot be interpreted in a meaning that no technical effect is achieved at higher concentrations.

45. The Board concludes that the embodiments attacked by the Appellants can also be considered as solving the above stated the technical problem.

Assessment of inventive step over document (8)

46. The skilled person, when reading document (8), would not get a hint that would encourage him to add to the reaction mixture concentrations of a thiol reagent other than CoA above 0.5mM/ml in order to solve the problem. Rather, when considering the results of table II on page 473, he would realize that the addition/omission of BSE has a much stronger influence on the light production by firefly luciferase than has the addition/omission of DTT.

47. It has further to be considered whether the skilled person starting from document (8) was led in an obvious manner by other prior art documents on file to solve the problem by choosing something falling within the claims.

48. Document (2), referring to the stimulation of luminescence by CoA, states on the top of page 522: "In recent experiments it has been found that thioethanolamine is slightly stimulatory." This statement does not allow any conclusion concerning the kind of stimulation and is not supported by any data. The paragraphs preceding the quoted sentence report of the very specific stimulatory effect of CoA.

49. In the passage bridging pages 525 to 526, document (2) reports that neither glutathione nor cysteine were found to react with adenyl-oxyluciferin as does CoA. This was found not to be surprising since CoA was highly specific in its effect on the stimulation of luminescence. Document (2) concludes that the secondary addition of cysteine or glutathione to a light reaction did not stimulate light emission. The Board does not consider that document (2) would lead the skilled man in any obvious manner to arrive at something falling within the claims.

50. Documents (6) and (12), both refer to CK-assays wherein the formation of ATP is measured using the luciferin-luciferase reaction in the presence of a thiol reagent other than CoA (see points (7) and (9) above). These documents, although considering the problem of improving the kinetics of light production of the luciferin-luciferase reaction, do not mention that the

added thiol reagents have an effect in this respect. The thiol reagents are added to the reaction mixture for their enzyme protective effect (document (12), column 3, lines 15 to 17). An improvement with regard to substantial consistency of the produced light signal is related to the addition of luciferase inhibitors, AMP in document (6) (see page 3, lines 57 to 62 and figure 1), L-luciferin in combination with an anti-CK-M antibody in document (12) (see column 2, lines 1 to 33).

51. Also document (7), relating to the automation of the luciferin-luciferase reaction, which implies that the production of a consistent light signal is aimed at, does not mention that mercaptoethanol, added to the reaction mixture, has an effect in this respect. However, the use of yet another luciferase inhibitor, namely $NaAsO_4$, is described on page 451, last paragraph.

52. Thus, it can be inferred from documents (6), (7) and (12) that the use of luciferase inhibitors take effect on the kinetics of light production of a beetle luciferase-luciferin reaction, in that, instead of a flash-like signal-time curve, a constant signal over a prolonged period of time is achieved. However, it is evident for the skilled person that this effect, caused by a partial inhibition of the enzyme is achieved at the cost of a reduced sensitivity of the enzymatic test.

53. Contrary to this, the solution of the underlying problem according to the patent in suit, i. e. the extension of the kinetics of light production by the addition of a thiol reagent other than CoA in the specified concentrations, allows the enzyme reaction to run with unchanged activity and thus does not reduce

the sensitivity of the analytic test. No suggestion of this effect can be derived by the skilled man from documents (6), (7) and (12).

54. Document (11) is directed to the possible use of the luciferin-luciferase reaction in genetic reporter assays (abstract), and is concerned with establishing optimal assay conditions to favour the long-lasting light production, thus allowing accurate measurements using a scintillation counter in the minutes which follow the mixing of the reagents (page 404, right column, last paragraph). The document proposes as a solution to this problem the use of high phosphate buffer concentrations, e. g. 100mM, which decreases but stabilises and prolongs the light emission (page 407, right column, last full paragraph). DTT is added to the assays at a concentration of 1mM to protect essential sulfhydryl groups (page 407, left column, first paragraph). There is nothing here directing the skilled man towards the inventions now claimed.

55. Document (4) discloses that luciferase could be protected from unspecific activation through addition of protecting substances such as BSE, thiol compounds and EDTA (column 6, lines 46 to 51). Figure 2 and the example in columns 7 and 8 show how luciferase inhibitors and pyrophosphate could be used so that a reagent with a stable light level is achieved already at a reasonable degree of inhibition. It is found that when using a reagent containing 10µg/ml L-luciferin and 10^{-6}M pyrophosphate, the decline of light emission as well as the initial peak are almost completely eliminated. This reagent is found to be suitable for analytical purposes. No effect in this respect

resulting from thiol compounds is mentioned in document (4).

56. In summary, none of the cited prior art documents, taken alone or in combination, contains technical information that would encourage a skilled reader trying to solve the problem underlying the patent in suit, to use a thiol reagent other than CoA in the concentrations required by independent claims 1, 14, 27, 33 and 40, in order to extend the kinetics of light production of a beetle luciferin-luciferase reaction.

57. The Board thus comes to the conclusion that none of the attacks on claims 1 to 42 succeed, and that the appeal must be dismissed.

Order

For these reasons it is decided:

The appeal is dismissed.

The Registrar: The Chairman:

P. Cremona S. C. Perryman