to 2.0 M, ii) a reducing agent, and iii) an alkalinising agent, thereby releasing the vitamin D compound from vitamin D-binding protein.

In a second aspect, the present invention concerns an in vitro method for measuring a vitamin D compound comprising the steps of a) providing a sample to be investigated, b) mixing the sample from step (a) with i) a reagent containing one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M, ii) a reducing agent, and iii) an alkalinising agent, thereby releasing a vitamin D compound from vitamin D-binding protein, and c) measuring the vitamin D compound released in step (b).

In a further embodiment the present disclosure concerns a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M and a reducing agent. In a third aspect, the present disclosure concerns use of a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M, and a reducing agent.

In a further embodiment the present disclosure concerns a reagent mixture comprising a sample to be investigated, a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M and a reducing agent, and an alkalinising agent. ~~In a fourth aspect, the present disclosure concerns a reagent mixture comprising a sample to be investigated, a reagent composition comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen~~

~~carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M, a reducing agent, and an alkalinising agent selected from the group consisting of NaOH, KOH, $Ca(OH)_2$ and LiOH, wherein the sample is blood, serum or plasma, and wherein the alkalinising agent has a concentration of 0.1 M to 2.0 M.~~

In a further embodiment the present disclosure concerns a kit for the release of a vitamin D compound from vitamin D-binding protein, which contains a reagent composition comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M and a reducing agent.

6. An in vitro method for measuring a vitamin D compound comprising the steps of:

   a) releasing a vitamin D compound from vitamin D-binding protein according to the method of any one of claims 1 to 5 and

   b) measuring the vitamin D compound released in step (a).

7. The method according to claim 6, wherein the vitamin D compound is selected from the group consisting of 25-hydroxyvitamin D_2, 25-hydroxyvitamin D_3, 24,25-dihydroxyvitamin D_2, 24,25-dihydroxyvitamin D_3 and C3-epi 25-hydroxyvitamin D.

8. The method according to claim 7, wherein the vitamin D compounds 25-hydroxyvitamin D_2 and/or 25-hydroxyvitamin D_3 are determined.

9. Use of a reagent composition for the release of a vitamin D compound from vitamin D-binding protein comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M, and a reducing agent.

10. Use of the reagent composition according to claim 9, characterized in that the reducing agent is selected from the group consisting of 2-Mercaptoethanol, 2-Mercaptoethylamine-HCl, TCEP, Cystein-HCl, Dithiothreitol (DTT), N-Methylmaleimide, Ellman's Reagent and 1,2-dithiolane-3-carboxylic acid.

11. Use of the reagent composition according to claim 9, characterized in that the reducing agent is selected from the group consisting of 2-Mercaptoethanol, 2-Mercaptoethylamine-HCl, TCEP, Cystein-HCl and Dithiothreitol (DTT).

12. Use of the reagent composition according to any one of claims ~~9~~ to 11, characterized in that the reducing agent has a concentration of 2 mM to 30 mM.

~~13. A reagent mixture comprising a sample to be investigated, a reagent composition comprising one hydrogen carbonate salt and a substance capable of releasing hydrogen carbonate ions (HCO_3^-) upon hydrolysis, wherein the total concentration of hydrogen carbonate ions (HCO_3^-) from the hydrogen~~

~~carbonate salt and released from the substance capable of releasing hydrogen carbonate ions (HCO_3^-) is 0.1 M to 2.0 M, a reducing agent, and an alkalinising agent selected from the group consisting of NaOH, KOH, $Ca(OH)_2$ and LiOH, wherein the sample is blood, serum or plasma, and wherein the alkalinising agent has a concentration of 0.1 M to 2.0 M.~~

~~14~~13. Use of the reagent composition according to any of claims 9 to 12 with an alkalinising agent selected from the group consisting of NaOH, KOH, $Ca(OH)_2$ and LiOH to release a vitamin D compound from vitamin D-binding protein, wherein the alkalinising agent has a concentration of 0.1 M to 2.0 M.