ASSAY METHOD AND KIT FOR ASSAY USE

TECHNICAL FIELD

This invention relates to an assay method and a kit for assay use, more particularly to an assay method in which a chromatography strip is used and a kit for assay use to be used in the assay method.

BACKGROUND ART

A method in which a chromatography strip is used is known as a quick and easy assay method. As described in Japanese Patent Application Kokai No. 60-19226, Japanese Patent Application Kokai No. 1-63865 and Japanese Patent Application Kokai No. 3-176659, the chromatography strip has a chromatography carrier in which, for example, a tracer comprised of a substance capable of binding specifically to an analyte , called trapping substance 1 in the present invention, and a marker is arranged in such a manner that its chromatographic development can be effected or in which a tracer is added at the time of the addition of a sample, and a substance capable of binding specifically to an analyte, called trapping substance 2 in the present invention, is immobilized on its downstream region. When a sample solution is added to a region where the tracer is arranged on the chromatography carrier or a tracer and a sample solution are added to the chromatography carrier at the same time, an analyte binds to the tracer, and the tracer to which the analyte is bound and the tracer to which the analyte is not bound are both developed in the longitudinal direction, namely downstream direction. When the tracer

reaches a region where the trapping substance 2 is immobilized, only the analyte (to which the tracer is bound) binds to the trapping substance 2 and the tracer is accumulated therein, while the tracer to which the analyte is not bound flows out in the downstream direction. Thus, the presence of the analyte and its quantity in the aqueous sample solution can be found by color development or the like at the region where the trapping substance 2 is immobilized.

In addition, in relation to the present invention, Japanese Patent Application Kokai No. 3-176659 describes about covering of the entire portion or a part of a region, called the marker region in the present invention, with gelatin.

Such a type of assay method in which a chromatography strip is used can perform the assay within a short period of time and its handling is easy, but it still requires improvements such as further increase in the detection sensitivity of analytes. In consequence, the problems to be resolved by the present invention are to provide an assay method in which a chromatography strip is used and detection sensitivity and the like are improved and to provide a kit for assay use which is used in the assay method in which detection sensitivity and the like are improved.

DISCLOSURE OF THE INVENTION

The inventors of the present invention have found that, when gelatin is added to a specified position of a chromatography carrier at the time of sample addition or arranged in advance on a specified position of the chromatography carrier, various points of the prior art assay methods and kits for assay use can be improved, for example,

the detection sensitivity is considerably increased, the detection time can be shortened and the pro-zone phenomenon in the sandwich method is reduced.

Accordingly, one aspect of the present invention is an assay method which uses a chromatography strip wherein its chromatography carrier has at least a binding region in which a trapping substance 2 capable of binding to an analyte is immobilized directly or indirectly via a trapping substance 3 capable of binding to both the analyte and the trapping substance 2 or in which a trapping substance 4 capable of binding specifically to a trapping substance 1 that binds specifically to the analyte or to the trapping substance 2 in competition with the analyte is immobilized at a downstream area of the region where the trapping substance 2 is immobilized, and a tracer comprised of the trapping substance 1 and a marker is arranged on an upstream area of said binding region in advance or added at the time of the addition of a sample, in a manner that the tracer can chromatographically develop; which comprises adding gelatin to an upstream area of said binding region at the time of the addition of a sample or arranging gelatin in advance in an upstream area of said binding region but excluding a region where the tracer is arranged in advance (to be referred to as marker region herein after).

Another aspect of the present invention is a kit for assay use which comprises at least the aforementioned chromatography strip and gelatin or a gelatin-arranged chromatography strip in which gelatin is arranged in advance in an upstream area of the binding region of the aforementioned chromatography carrier but excluding the marker

region.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is an illustration describing the chromatography strip.

Fig. 2 is a graph showing results of an assay carried out by the assay method of the present invention (Example 2).

Fig. 3 is a graph showing results of an assay carried out by the assay method of the present invention (Example 3).

BEST MODE OF CARRYING OUT THE INVENTION

The analyte whose presence or amount in a sample is detected by the present invention is not particularly limited, with the proviso that any compound which binds specifically to the analyte is present. Its examples include a compound in which biotin is introduced (to which avidine binds specifically), a compound which contains avidine, complementary DNA, a compound or hapten which has antigenicity, a compound which binds specifically to antibodies of a certain antigen, an antibody, an antibody fragment which binds specifically to its antigen, lectin which binds specifically to a sugar ligand and a sugar ligand which binds specifically to lectin, as well as a ligand which binds specifically to a receptor and a receptor which binds specifically to a ligand. As a matter of course, the detection sensitivity becomes high when these binding reactions have high reactivity or affinity. Their illustrative examples include microorganisms; viruses; antigens such as specific antigens of pathogenic microorganisms, viruses and the like and tumor marker antigens of prostatic cancer and the like; antibodies such as those which are induced by microbial, viral and the like infections,

autoantibodies and the like antibodies and antibodies specific for acute phase protein antigens and the like; fecal hemoglobin or hemoglobin-specific antigen; cells such as a cell which brings up a specific antigen, a cell which has a specific sugar ligand and a cell which has a specific receptor; hormones such as thyroid hormone, parathyroid hormone, gonad stimulating hormone and the like and physiologically active proteins or peptides such as cytokine, monokine and the like; proteins or glycoproteins such as enzymes, proteoglycan, γ-seminoprotein, amyloid precursors and the like; low molecular chemical substances or drugs in blood; signal transfer controlling factors such as agonists, antagonists and the like; thrombolysis/blood coagulation controlling factors; gene expression controlling factors; cell adhesion molecules; lectins such as immunomodulator lectin and the like; and RNA, DNA and the like nucleic acids. Particularly, feces, urine and the like are desirable as non-invasive samples.

The trapping substance 1 is comprised of a compound which binds specifically to an analyte by trapping it or which performs specific binding to (trapping of) a trapping substance 2 which will be described later, in competition with an analyte. That is, when the analyte is an antigen, the trapping substance 1 is an antibody or an antibody fragment which binds specifically to the antigen (hereinafter, the term "antibody" includes antibody fragments which bind specifically to the antigen) or an antigen or a compound which binds to antibody specifically (hereinafter, the term "antigen" includes compounds which bind to antibody specifically), and is an antigen or antibody

when the analyte is an antibody. Also, when the analyte is a ligand (or a compound having a ligand), the trapping substance 1 is a receptor (or a compound having a receptor) or a ligand, and it is a ligand or a receptor when the former is a receptor. In some cases, the trapping substance 1 performs specific binding to a trapping substance 3 via an analyte, thereby resulting in its binding to the trapping substance 2 via the trapping substance 3. Binding of an analyte with the trapping substance 1 may be either reversible or irreversible, but the detection sensitivity decreases when the affinity is too low.

A marker is added to the trapping substance 1 to form a tracer. Addition of a marker to the trapping substance 1 may be effected by absorption or inclusion of the marker with the trapping substance 1 or by their binding through hydrogen bonding or covalent bonding. In short, a marker may be added in such a manner that the trapping substance 1 is not easily released therefrom and can be recognized by an analyte or the trapping substance 2.

When the trapping substance 1 binds to an analyte, the trapping substance 2 which will be described later or the trapping substance 4 which will also be described later, the marker generates a signal, namely a color, which indicates their presence. Preferably, the marker may be composed of microparticles which are insoluble in water and have poor water repellency. Examples of the desirable markers include gold colloid and the like metal colloids and selenium colloid and the like non-metal colloids; organic colloids; dyestuff particles; colored polystyrene and the like colored organic polymers; and liposomes and the like oily microparticles in which a coloring

agent is encapsulated. When colloids and the like do not develop color, a coloring agent is added. Examples of the coloring agent include those which develop color at the visible region, as well as fluorescent materials and substances which develop color at the ultraviolet region. Substances which develop color, including fluorescence, by enzymes and the like arranged in a binding region or the like are also included in the coloring agent of the present invention.

The tracer, either by itself or in a bound form to an analyte, develops and travels in the chromatography carrier when a sample solution is added to the chromatography strip. The tracer is used as a solution (or a suspension), but can be preserved in a powder form by a freeze-drying or the like means.

There are two methods for arranging the tracer in such a manner that its chromatographic development can be effected on the chromatography carrier with an aqueous solvent. In the first method, a section of the chromatography carrier which has been impregnated with a tracer aqueous solution and then dried is arranged in advance on a base material or the chromatography carrier at an upstream position of a binding region which will be described later, by making an appropriate distance from the binding region. In this case, the position where the tracer is arranged is called marker region. In the second method, the tracer is added to the chromatography carrier at the time of the addition of a sample. In this case, therefore, the chromatography strip does not have a marker region.

The trapping substance 2 binds to the tracer which has an analyte via the analyte, or to the tracer which has the trapping substance

1 via the trapping substance 1. In the latter case in which its binding is effected via the trapping substance 1, it binds to the trapping substance 2 in competition with the analyte. In either case, the trapping substance 2 binds to the analyte specifically. In another mode, it may be bound to an analyte indirectly by its specific binding to the trapping substance 3 which binds specifically to the analyte. An illustrative example of the trapping substance 2 is an antibody (as defined in the foregoing, the term "antibody" as used herein also include an antibody fragment which binds to the antigen specifically) when the analyte is an antigen. When the trapping substance 2 is an antibody and the trapping substance 1 is also an antibody, it is desirable that they recognize different epitopes of the antigen to be detected. In the same manner, it is an antigen (as defined in the foregoing, the term "antigen" as used herein also include a compound which binds to an antibody specifically) when the analyte is an antibody, the former is a ligand when the latter is a receptor and the former is a receptor when the latter is a ligand. In addition, when the trapping substance 2 binds to the trapping substance 3 specifically, avidine, anti-biotin antibody, lectin and the like can be exemplified as the trapping substance 2.

Immobilization of the trapping substance 2 on the chromatography carrier may be effected either by covalent bonding between an appropriate functional group of the chromatography carrier and a functional group of the trapping substance 2 or by adsorbing the trapping substance 2 strongly to the chromatography carrier. Alternatively, the trapping substance 2 may be bound or adsorbed to

microparticles which are then dispersed and immobilized in the chromatography carrier. In this connection, the term immobilization means arrangement of a substance in such a manner that it is not substantially dispersed or developed by aqueous solvents. The area where the trapping substance 2 is immobilized is called binding region.

When the trapping substance 1, trapping substance 2, trapping substance 3 or trapping substance 4 is an antibody, a monoclonal antibody is used in most cases, but even a serum antibody can be used by if necessary carrying out appropriate means such as absorption treatment, selection of the antigen or animal species and the like.

The trapping substance 3 is a conjugate body which comprises a portion that binds to an analyte specifically and another portion that binds to the trapping substance 2 specifically. As described in detail in relation to the trapping substance 1, the portion that binds to an analyte specifically is an antigen when the analyte is an antibody, an antibody when the latter is an antigen, a receptor when the latter is a ligand or a ligand when the latter is a receptor. The portion that binds to the trapping substance 2 specifically is, for example, biotin when the trapping substance 2 is avidine, anti-biotin antibody or avidine when the latter is biotin or a sugar ligand when the latter is lectin.

The trapping substance 3 is arranged in advance on any optionally selected region in the upstream area of the binding region of the chromatography carrier, in such a manner that it can develop and travel in the chromatography carrier when an aqueous sample solution is added to the chromatography strip, or is added to the

chromatography carrier at the time of the addition of the sample. The arranging method is as described in detail in relation to the arrangement of the tracer.

The trapping substance 4 binds to the trapping substance 1 specifically.

The chromatography carrier is not particularly limited, provided that it can develop solutes with an aqueous solvent. For example, it may be comprised of non-woven fabric made of cellulose, nitrocellulose, cellulose acetate, polyacrylate, glass, agarose, polyurethane or the like or a combination thereof with an inorganic powder.

The chromatography strip is obtained for example by laminating the chromatography carrier on an appropriate base material when it is necessary.

As is universally known, gelatin is obtained from vertebrates such as fishes, mammals and the like. Melting temperature of the gelatin gel is preferably 15°C or more, more preferably 25°C or more.

According to the present invention, melting temperature of the gelatin gel is measured by the following method.

Gelatin is dissolved in 10 mM phosphate buffer (pH 7.4) containing 0.9% sodium chloride to a final concentration of 10 g/dl. A 1 ml portion of the thus prepared gelatin solution is put into a test tube of 100 mm in length and 12.5 mm in diameter, and the test tube is sealed with a stopper. The test tube is fully soaked in a water bath of 0°C to effect gelation of gelatin. Thereafter, temperature of the water bath is slowly increased at a rate of 2 minutes

per 1°C to measure the first gelatin gel-dropping temperature when the test tube is turned upside down.

Gelatin is used when a sample solution is added or arranged in advance on the chromatography carrier. When used at the time of the sample addition, it may be dissolved in the sample solution or added separately from the sample solution as an aqueous gelatin solution. As the aqueous solvent for use in the preparation of the aqueous gelatin solution, it is desirable in general to use the same aqueous solvent used in the preparation of the sample solution. When gelatin is used at the time of the sample addition, concentration of gelatin in the gelatin solution is preferably within the range of from 0.1 to 1.5% by weight. When gelatin is arranged in advance, it is desirable also to arrange gelatin in advance in an amount of the aforementioned weight % per sample solution.

When gelatin is added at the time of the addition of a sample solution, it is added to a position upstream of the binding region. When gelatin is arranged in advance, it is arranged at a position upstream of the binding region but excluding the marker region. As the illustrative adding or arranging position, the most suitable position is optionally selected in advance, and the most excellent results are obtained in some cases when arranged on the region 7. When a gelatin-arranged chromatography strip in which gelatin is arranged on the chromatography carrier in advance is obtained, it is arranged on a position selected in advance within the upstream area of the binding region but excluding the marker region in such a manner that it can be developed with an aqueous solvent. That is, a section of

the chromatography carrier which has been impregnated with a gelatin aqueous solution and then dried is arranged on a base material or the chromatography carrier.

Also, when the trapping substance 4 which binds to the trapping substance 1 specifically is immobilized on the downstream area of the chromatography carrier where the trapping substance 2 is bound, in order to prepare the binding region, it is desirable to add or arrange in advance gelatin to or on an area between the region where the trapping substance 2 is immobilized and the region where the trapping substance 4 is immobilized.

When gelatin is used by other methods than those of the present invention, for example, when gelatin is arranged on the marker region in advance, it causes prolonged detection time, decreased detection sensitivity and the like problems in many cases. On the other hand, when gelatin is used for example at the time of the sample addition, such problems do not occur even if it is added to the marker region. The reason for this is that, when gelatin is arranged on the marker region in advance, gelatin exerts certain influence upon the marker region while preservation of the chromatography strip.

The aqueous solvent of the sample solution may be water, but a pH buffer is generally used. The aqueous solvent may have a pH value within the range of preferably from 5 to 9.

The sample solution is added to the marker region or an upstream area of the region when the marker region is present or to an upstream area of the binding region when the marker region is not present. The term addition as used herein means a case in which the sample solution

is put on the chromatography strip or a case in which the most upstream tip of the chromatography strip is soaked in the sample solution.

When the tracer and/or trapping substance 3 is not arranged on the chromatography carrier in advance, these may be added at the time of the sample addition. With regard to their adding method, they are usually added together with the sample.

The kit for assay use of the present invention includes two cases.

In the first case, at least a chromatography strip having no gelatin arranged in advance and gelatin are used in combination. In this case, an aqueous solvent and the like may be further used in combination. Also, gelatin may be dissolved or not dissolved in the aqueous solvent.

The second case is composed of a gelatin-arranged chromatography strip. Also in this case, an aqueous solvent and the like other constituting components may be used in combination. In either of the above cases, the tracer and/or trapping substance 3, when not arranged on the chromatography carrier in advance, may be used in the form of powder prepared by freeze-drying or the like means or as an aqueous solution (or a suspension).

The present invention is further described with reference to the drawings.

Fig. 1 represents a chromatography strip in which the marker region is arranged. The chromatography strip 1 is composed of the chromatography carrier 2 and, as occasion demands, a base material. The chromatography carrier 2 has the marker region 3 which is arranged

in such a manner that the tracer can be developed with an aqueous solvent.

The upstream region 5 of the marker region 3 is arranged as occasion demands and becomes the adding position of sample solutions in many cases when arranged. This region can perform filtration of suspended materials in the sample solution and also can contain auxiliary substances effective in carrying out the assay smoothly. Examples of such auxiliary substances include those which can make the binding reaction of an analyte with the trapping substance 1 more specific or quantitative, as well as coloring reagents of insoluble markers and the like.

When a sample solution is added to an upstream area of the marker region 3 or to the marker region 3, an analyte contained in the sample, if it can bind to the trapping substance 1, is labeled by the tracer. Next, each of the tracer to which the analyte is bound, the remaining tracer to which the analyte is not bound and the remaining analyte travels by the chromatographic development toward the right-side direction in Fig. 1, namely the downstream direction, and reaches the binding region 4 arranged on a downstream area of the marker region 3.

The marker region 3 and the binding region 4 are not connected directly to each other but arranged by keeping a certain space which is optionally selected. The region 7 which is used to make this space may have gelatin put thereon or the marker substance 3 which is arranged thereon in such a manner that it can perform chromatographic development. In some cases, a coloring agent of the marker may be

further arranged thereon.

The binding region 4 includes two cases. That is, in the first case, the tracer which bound with an analyte is trapped in this region and develops color, or the tracer competes with the analyte and is trapped in this region to develop color. In the second case, the trapping substance 4 is immobilized on a downstream area of a region where the trapping substance 2 is immobilized (this region corresponds to the binding region in the aforementioned first case). In this case, the arrangement is made optionally as occasion demands, but when arranged and used for the detection of color development, it is used as the binding region 4. In the second case, the tracer which is not bound to and trapped by the trapping substance 2 through its competition with an analyte is trapped by the trapping substance 4 in this binding region and develops color.

When the trapping substance 3 is arranged, the trapping substance 3 binds to an analyte which is bound with the tracer, and the tracer/analyte/trapping substance 3 complex formed thereby binds to the trapping substance 2 via the trapping substance 3 and stops at the binding region 4. Alternatively, the trapping substance 3 binds to the trapping substance 2 and then binds to an analyte specifically in the binding region 4.

The downstream region 6 of the binding region 4 is arranged as occasion demands, in order to flow out the tracer to which an analyte is not bound via the trapping substance 1 when the trapping substance 1 is able to bind with the analyte, or to flow out soluble components other than the analyte contained in the sample solution. In some cases,

a substance which develops color when the aqueous solvent of an aqueous sample solution is flowed in, such as a pH indicator or the like, is arranged on the downstream region 6, thereby rendering possible judgment of the detection reliability.

When the chromatography strip does not have the marker region 3, the assay is carried out in the same manner as described in the foregoing, except that the tracer is added at the time of the sample addition.

Example 1

Preparation of tracer composed of anti-human hemoglobin antibody labeled with selenium colloid:

Selenium colloid was prepared by stirring 91 mM L-sodium ascorbate and 32 mM selenium oxide at about 4°C for 15 minutes and then at about 42°C for about 70 hours.

The thus obtained selenium colloid was diluted with 10 mM bis-tris buffer solution (pH 7.0) to such a concentration that its absorbance at 550 nm became 15. Anti-human hemoglobin mouse monoclonal antibody (0.02%) was added to the diluted preparation, and the mixture was stirred at room temperature for 1 hour. Next, the thus obtained anti-human hemoglobin antibody labeled with selenium colloid was washed with 10 mM Tris-HCl buffer solution (pH 7.2) and used as a tracer.

Preparation of marker region:

A tracer suspension was obtained by adding the tracer to 10 mM Tris-HCl buffer solution (pH 7.2) containing 1% casein, and adjusting absorbance of the mixture to 1.0 at a wave length of 550

nm. A glass fiber membrane (Lypore 9524, manufactured by LYDALL, U.S.A.) was soaked in the thus prepared suspension to thoroughly impregnate it with the suspension and then the glass fiber membrane was dried to be used as a marker region.

Preparation of binding region:

Anti-human hemoglobin mouse monoclonal antibody having a binding site which is different from that of the aforementioned anti-human hemoglobin antibody was mixed with 30 mM Tris-HCl buffer (pH 7.4) containing 150 mM sodium chloride, to a final concentration of 2 mg/ml. Separately from this, a laminate (PET100 PE LR007A, manufactured by Lintech) having a thickness of 100 μm and a rectangular size of 0.4 x 6.0 cm was adhered to a nitrocellulose membrane (manufactured by Schleicher & Schuell, U.S.A.) used as a chromatography carrier having a rectangular size of 0.4 x 4.5 cm and a pore size of 5 μm, in such a manner that their upper ends matched when their longer sides were arranged lengthways. The antibody-mixed solution was spotted in a line on a region about 1 cm from the bottom of the nitrocellulose film to which the laminate was adhered, and then the spots were thoroughly dried to immobilize the anti-human hemoglobin antibody.

Preparation of chromatography strip:

A pad cut into a square size of 0.4 x 0.4 cm containing the selenium colloid-labeled anti-human hemoglobin antibody (marker region) was adhered on the laminate, under side of the nitrocellulose in which the anti-human hemoglobin antibody has been immobilized, in such a manner that it slightly contacted with the nitrocellulose. As

a pre-filter, hydrophobic non-woven fabric (Sontala 8801, manufactured by Du Pont, U.S.A.) cut into a size of 0.4 x 1.3 cm was adhered on the laminate under side of the pad, in such a manner that it slightly contacted with the nitrocellulose, thereby obtaining a chromatography strip.

Assay:

0.1 M Tris-HCl Buffer (pH 7.6) containing 0.1% bovine serum albumin (manufactured by Seikagaku Kogyo, Tokyo), 0.9% sodium chloride and 0.1% sodium azide, further supplemented with human hemoglobin (manufactured by Sigma, U.S.A.), was used as the sample. To this was added swine skin gelatin (manufactured by Sigma, U.S.A.) to a final concentration of 0.7%. A 25 µl portion of the sample solution was applied on the pre-filter of the chromatography strip. After 7 minutes of the sample addition, the result was judged by observing selenium colloid-originated "redness" with the naked eye. The minimum concentration of hemoglobin by which the "redness" can be observed with the naked eye was used as the sensitivity.

Results of the judgment are shown in Table 1. As is evident from Table 1, the sensitivity increased by a factor of about 80 times when gelatin was used (a) in comparison with a case in which gelatin was not used (b).

Table 1

	Human hemoglobin concentration (ng/ml)										
	0	0.156	0.313	0.625	1.25	2.5	5.0	10	25	50	100
a	-	-	+	+	+	+	+	+	+	+	+
b	-	-	-	-	-	-	-	-	+	+	+

Example 2

The procedure of Example 1 was repeated, except that the judgment was started 1 minute after the sample addition until 12 minutes thereafter at 1 minute intervals.

The results are shown in Fig. 2. As is evident from Fig. 2, the assay time was shortened to about 1/3 when gelatin was used (line a) in comparison with a case in which gelatin was not used (line b).

Example 3

The procedure of Example 1 was repeated, except that the concentration of human hemoglobin in the sample solution was changed to the values shown in Fig. 3. The results are shown in Fig. 3. As is evident from Fig. 3, the upper limit of measurable human hemoglobin concentration when gelatin was used (line a) is about 10 times higher than the measurable upper limit when gelatin was not used (line b). In other words, it is evident that the pro-zone phenomenon is reduced when gelatin is used.

Example 4

The procedure of Example 1 was repeated, except that human hemoglobin was detected using gelatin preparations shown in Table 2.

In this case, the concentration of human hemoglobin was fixed to 10 ng/ml, and the amount of gelatin to be added to the sample solution was changed to 1.0%. The results are shown in Table 2. The melt temperature of gelatin was measured by the method described in the foregoing.

Table 2

	Gelatin gel melt temperature (°C)	Human hemoglobin detection result
Swine skin gelatin (Sigma)	31.4	+++
Bovine skin gelatin (Sigma)	24.9	+
Swine bone gelatin (Difco)	27.5	+
Cold fish gelatin (Sigma)	0.0	±
No addition		-

INDUSTRIAL APPLICABILITY

By the use of the assay method or assay kit of the present invention, detection sensitivity of analytes can be increased. Also, the time required for their detection can be shortened. In addition, so-called pro-zone phenomenon in the sandwich method is reduced.

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CLAIM

1. An assay method which uses a chromatography strip wherein its chromatography carrier has at least a binding region in which a trapping substance 2 capable of binding to an analyte is immobilized directly or indirectly via a trapping substance 3 capable of binding to both the analyte and the trapping substance 2 or in which a trapping substance 4 capable of binding specifically to a trapping substance 1 that binds specifically to the analyte or to the trapping substance 2 in competition with the analyte is immobilized at a downstream area of the region where the trapping substance 2 is immobilized, and a tracer comprised of the trapping substance 1 and a marker is arranged on an upstream area of said binding region in advance or added at the time of the addition of a sample, in a manner that the tracer can chromatographically develop; which comprises adding gelatin to an upstream area of said binding region at the time of the addition of a sample or arranging gelatin in advance in an upstream area of said binding region but excluding a region where the tracer is arranged in advance.

2. The assay method according to claim 1 wherein the gelatin gel melts at 15°C or more.

3. The assay method according to claim 1 wherein the gelatin gel melts at 25°C or more.

4. The assay method according to claim 1, 2 or 3 wherein the sample is feces or urine.

5. The assay method according to claim 1 wherein, when a region where the trapping substance 4 capable of binding specifically

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to the trapping substance 1 is immobilized in a downstream area of a region where the trapping substance 2 is immobilized is used as the binding region, gelatin is added or arranged in advance to or on an area between the region where the trapping substance 2 is immobilized and the region where the trapping substance 4 is immobilized.

6. A kit for assay use which comprises at least the chromatography strip of claim 1 and gelatin or a gelatin-arranged chromatography strip in which gelatin is arranged in advance in an upstream area of the binding region of claim 1 but excluding a region where the tracer is arranged in advance.

7. The kit for assay use according to claim 7 wherein the gelatin gel melts at 25°C or more.

8. The kit for assay use according to claim 7 wherein the gelatin gel melts at 15°C or more.

9. The kit for assay use according to claim 6, 7 or 8 wherein the sample is feces or urine.

10. The kit for assay use according to claim 1 wherein, when a region where the trapping substance 4 capable of binding specifically to the trapping substance 1 is immobilized in a downstream area of a region where the trapping substance 2 is immobilized is used as the binding region, gelatin is added or arranged in advance to or on an area between the region where the trapping substance 2 is immobilized and the region where the trapping substance 4 is immobilized.