reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0011] To meet these and other demands, provided herein are methods for generating a solid antigen/carrier protein gel. These solid gels can be processed like tissue samples or other biological samples according to standard IHC or electron microscopy (EM) processing methods (including fixation, sectioning, antigen retrieval, and so forth). Since the gels contain a known amount of an antigen of interest, they can be used, *e.g.*, to create a series of gels having known concentrations of antigen to standardize staining with a particular antibody, or to screen for antibodies that specifically recognize an antigen of interest and are suitable for IHC/EM analyses. These methods are thought to provide a general platform that allows for control staining of any antigen of interest, simulating multiple levels of expression using known concentrations of antigen.

[0012] Certain examples of the present disclosure relate to methods of generating a solid antigen/carrier protein gel (*e.g.*, for IHC or EM analysis) comprising: (a) mixing a purified antigen with a liquid solution comprising a carrier protein selected from the group consisting of an albumin protein (*e.g.*, a serum albumin protein), an egg white protein or a mixture of egg white proteins, and gelatin to produce an antigen/carrier protein liquid solution; and (b) heating the antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel. In some examples, the methods comprise: (a) mixing a purified antigen with a liquid solution comprising a carrier protein selected from the group consisting of an albumin protein (*e.g.*, a serum albumin protein), an egg white protein or a mixture of egg white proteins, gelatin, and poly-lysine to produce an antigen/carrier protein liquid solution; and (b) heating the antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel. In some examples, the methods further comprise, after (b): dehydrating the solid antigen/carrier protein gel and embedding the dehydrated solid antigen/carrier protein gel in a paraffin block. In some examples, the methods further comprise, after embedding the dehydrated solid antigen/carrier protein gel in the paraffin block: transferring a core comprising the antigen/carrier protein gel from the paraffin block to a recipient tissue microarray (TMA) block. In some examples, the methods further comprise, after (b): incubating the solid

antigen/carrier protein gel in a liquid embedding medium and freezing the solid antigen/carrier protein gel in the embedding medium. In some examples, the methods further comprise, after (b): embedding the solid antigen/carrier protein gel in a plastic resin. In some examples, the methods further comprise, after (b): sectioning the solid antigen/carrier protein gel into one or more solid antigen/carrier protein gel sections having a thickness of between about 30nm and about 50µm. In some examples, the solid antigen/carrier protein gel is sectioned into one or more solid antigen/carrier protein gel sections having a thickness of between about 2µm and about 30µm. In some examples, the solid antigen/carrier protein gel is sectioned into one or more solid antigen/carrier protein gel sections having a thickness of between about 30nm and about 100nm. In some examples, the methods further comprise, prior to (b), including a fixative in the antigen/carrier protein liquid solution. In some examples, the fixative comprises formaldehyde. In some examples, the antigen/carrier protein liquid solution comprises formaldehyde at a final concentration of at least about 1%. In some examples, the fixative comprises glutaraldehyde, Davidson's fixative, Bouin's fixative, ½ strength Karnovski's fixative, or a zinc salt. In some examples, the methods further comprise subjecting the solid antigen/carrier protein gel to antigen retrieval. In some examples, subjecting the solid antigen/carrier protein gel to antigen retrieval comprises heating the solid antigen/carrier protein gel in a liquid solution. In some examples, the methods further comprise: prior to (b), including a fixative in the antigen/carrier protein liquid solution; after (b), dehydrating the solid antigen/carrier protein gel; embedding the dehydrated solid antigen/carrier protein gel in a paraffin block; sectioning the paraffin block with the embedded antigen/carrier protein gel into one or more sections having a thickness of between about 30nm and about 50µm; subjecting the one or more sections of the embedded solid antigen/carrier protein gel to antigen retrieval; and after antigen retrieval, blocking the one or more sections of the embedded solid antigen/carrier protein gel.

In some examples, the methods further comprise: after (b), incubating the solid antigen/carrier protein gel in a liquid embedding medium; freezing the solid antigen/carrier protein gel in the embedding medium; sectioning the frozen antigen/carrier protein gel into one or more sections having a thickness of between about 30nm and about 50µm; and blocking the one or more sections of the frozen antigen/carrier protein gel. In some examples, the antigen is a polypeptide antigen. In some examples, the antigen comprises an N-terminal tyrosine, a C-terminal cysteine, or

both. In some examples, the methods further comprise prior to (b), cross-linking the antigen with the carrier protein (*e.g.*, albumin protein, egg white protein or mixture of egg white proteins, or gelatin) using a cysteine-reactive reagent. In some examples, the antigen comprises a non-polypeptide antigen. In some examples, the carrier protein is an albumin protein, such as bovine, goat, horse, or human serum albumin. In some examples, the carrier protein is an egg white protein or a mixture of egg white proteins. In some examples, the antigen/carrier protein liquid solution produced in (a) comprises the carrier protein at a concentration of greater than or equal to 2% (w/v). In some examples, the antigen/carrier protein liquid solution produced in (a) comprises the carrier protein at a final concentration of less than or equal to about 25% (w/v). In some examples, the carrier protein is gelatin, and the method further comprises, after (b), cooling the heated antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel. In some examples, the method further comprises, after cooling the heated antigen/carrier protein liquid solution: incubating the solid antigen/carrier protein gel with a fixative to form a fixed antigen/carrier protein gel; and dehydrating the fixed antigen/carrier protein gel. In some examples, the antigen/carrier protein liquid solution produced in (a) comprises the carrier protein at a concentration of greater than or equal to about 0.5% (w/v). In some examples, the antigen/carrier protein liquid solution is heated in (b) to at least about 65°C. In some examples, the antigen/carrier protein liquid solution is heated in (b) to at least about 65°C for at least 6 minutes. In some examples, the poly-lysine is present in the antigen/carrier protein liquid solution produced in (a) at a concentration of about 14mg/mL.

[0012a] In one aspect, the present disclosure refers to a method for generating a solid antigen/carrier protein gel for immunohistochemical (IHC) staining, the method comprising:

(a) mixing a purified antigen with a liquid solution comprising a carrier protein to produce an antigen/carrier protein liquid solution; and

(b) heating the antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel,

wherein the carrier protein consists of a serum albumin protein and the antigen/carrier protein liquid solution further comprises formaldehyde.

[0013] Further provided herein are solid antigen/carrier protein gels produced by the method according to any one of the above examples. In one aspect, the present disclosure refers to a solid antigen/carrier protein gel for immunohistochemical (IHC) staining comprising a purified antigen cross-linked to a carrier protein, wherein the carrier protein consists of a serum albumin protein and the solid gel has been fixed in a fixative comprising formaldehyde.

[0014] Further provided herein are tissue microarrays (TMAs) comprising at least a first solid antigen/carrier protein gel produced by the method according to any one of the above examples and a second solid antigen/carrier protein gel produced by the method according to any one of the above examples.

[0015] Certain examples of the present disclosure relate to methods for immunohistochemical (IHC) staining of an antigen, comprising: providing a solid antigen/carrier protein gel produced by the method of any one of the above examples, wherein the solid antigen/carrier protein gel contains the antigen; providing a sample; contacting the solid antigen/carrier protein gel

and the sample with a primary antibody that specifically binds the antigen; after contacting the solid antigen/carrier protein gel and the sample with the primary antibody, contacting the solid antigen/carrier protein gel and the sample with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody; detecting a signal of the detectable moiety from the solid antigen/carrier protein gel; and detecting a signal of the detectable moiety from the sample, wherein detection of a signal from the sample as compared to the signal detected from the solid antigen/carrier protein gel indicates the presence of the antigen in the sample. In some examples, the detectable moiety comprises an enzyme, and wherein detecting the signal of the detectable moiety comprises exposing the detectable moiety to a chromogenic, fluorescent, or chemiluminescent substrate of the enzyme and detecting a signal from the substrate upon reaction with the enzyme. In some examples, the detectable moiety comprises a fluorophore, metal particle, metal ion, radioisotope, nucleic acid, electrochemiluminescent reporter, or a "quantum dot" (a solid state, semiconductor or carbon-based fluorescent nanoparticle). In some examples, the sample is a tissue sample.

[0016] Certain examples of the present disclosure relate to methods for control immunohistochemical (IHC) staining of an antigen, comprising: providing a first and a second solid antigen/carrier protein gel, wherein each of the first and the second solid antigen/carrier protein gels is produced by the method of any one of the above examples, wherein the first solid antigen/carrier protein gel contains the antigen at a first concentration, and wherein the second solid antigen/carrier protein gel contains the antigen at a second concentration higher than the first concentration; contacting the first and the second solid antigen/carrier protein gels with a primary antibody that specifically binds the antigen; after contacting the first and the second solid antigen/carrier protein gels with the primary antibody, contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody; detecting a first signal of the detectable moiety from the first solid antigen/carrier protein gel; and detecting a second signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of a second signal greater than the first signal indicates control IHC staining of the antigen. In some examples, the detectable moiety comprises an enzyme, and wherein detecting the signal of the detectable moiety comprises exposing the

detectable moiety to a chromogenic, fluorescent or chemiluminescent substrate of the enzyme and detecting a signal from the substrate upon reaction with the enzyme. In some examples, the detectable moiety comprises a fluorophore, metal particle, metal ion, radioisotope, nucleic acid, electrochemiluminescent reporter, or a "quantum dot" (a solid state, semiconductor or carbon-based fluorescent nanoparticle). In some examples, the first concentration is 0nM, and wherein detection of a lack of a first signal indicates control IHC staining of the antigen. In some examples, the methods further comprise: providing a sample; contacting the sample with the primary antibody; after contacting the sample with the primary antibody, contacting the sample with the secondary antibody, wherein a detectable moiety is conjugated to the secondary antibody; detecting a third signal of the detectable moiety from the sample; and comparing the third signal with the first and the second signals, wherein an amount of the third signal relative to the amounts of the first and the second signals indicates an abundance of the antigen in the sample, relative to the amounts of the antigen in the first and the second solid antigen/carrier protein gels.

[0017] Certain examples of the present disclosure relate to methods for control immunohistochemical (IHC) staining with a secondary antibody, comprising: providing a first and a second solid antigen/carrier protein gel, wherein each of the first and the second solid antigen/carrier protein gels is produced by the method of any one of the above examples, wherein the first solid antigen/carrier protein gel comprises a first antibody having a first isotype, and wherein the second solid antigen/carrier protein gel comprises a second antibody having a second isotype different from the first isotype; contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the first isotype, wherein a detectable moiety is conjugated to the secondary antibody; detecting a signal of the detectable moiety from the first solid antigen/carrier protein gel; and detecting a lack of the signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of the signal associated with the first solid antigen/carrier protein gel and the lack of the signal associated with the second solid antigen/carrier protein gel indicates control staining with the secondary antibody. In some examples, the detectable moiety comprises an enzyme, and wherein detecting the signal of the detectable moiety comprises exposing the detectable moiety to a chromogenic, fluorescent or chemiluminescent substrate of the enzyme and detecting a signal from the substrate upon reaction with the enzyme. In some

examples, the detectable moiety comprises a fluorophore, metal particle, metal ion, radioisotope, nucleic acid, electrochemiluminescent reporter, or a "quantum dot" (a solid state, semiconductor or carbon-based fluorescent nanoparticle).

[0018] Certain examples of the present disclosure relate to solid antigen/carrier protein gels (*e.g.*, for immunohistochemical (IHC) staining) comprising a purified antigen cross-linked to a carrier protein selected from the group consisting of an albumin protein (*e.g.*, a serum albumin protein), an egg white protein or a mixture of egg white proteins, and gelatin. In some examples, the present disclosure relates to solid antigen/carrier protein gels (*e.g.*, for immunohistochemical (IHC) staining) comprising a purified antigen cross-linked to a carrier protein selected from the group consisting of an albumin protein (*e.g.*, a serum albumin protein), an egg white protein or a mixture of egg white proteins, gelatin, and poly-lysine. In some examples, the solid gel has a thickness of between about 30nm and about 50µm. In some examples, the solid gel has a thickness of between about 2µm and about 30µm. In some examples, the solid gel has a thickness of between about 30nm and about 100nm. In some examples, the solid gel is frozen in an embedding medium. In some examples, the solid gel is embedded in paraffin. In some examples, the solid gel is embedded in a plastic resin. In some examples, the solid gel is affixed to a solid substrate. In some examples, the solid gel has been fixed in a fixative. In some examples, the fixative comprises formaldehyde. In some examples, the fixative comprises formaldehyde at a concentration of at least about 1%. In some examples, the fixative comprises glutaraldehyde, Davidson's fixative, Bouin's fixative, ½ strength Karnovski's fixative, or a zinc salt. In some examples, the solid gel has been subjected to antigen retrieval. In some examples, the antigen is a polypeptide antigen. In some examples, the antigen comprises an N-terminal tyrosine, a C-terminal cysteine, or both. In some examples, the N-terminal tyrosine and/or C-terminal cysteine is cross-linked to the carrier protein. In some examples, the antigen comprises a non-polypeptide antigen. In some examples, the carrier protein is an albumin protein, such as bovine, goat, horse, or human serum albumin. In some examples, the carrier protein is an egg white protein or a mixture of egg white proteins. In some examples, the solid gel comprises the carrier protein at a concentration of greater than or equal to 2%. In some examples, the solid gel comprises the carrier protein at a concentration of less than or equal to about 25%. In some examples, the carrier

protein is gelatin. In some examples, the solid gel comprises the carrier protein at a concentration of greater than or equal to 0.5%. In some examples, the solid gel contains the antigen at a concentration of at least about 25nM. In some examples, the solid gel comprises poly-lysine at a concentration of about 14 mg/mL.

[0019] Certain examples of the present disclosure relate to tissue microarrays (TMAs) comprising at least a first and a second solid antigen/carrier protein gel, wherein the first and second solid antigen/carrier protein gels both comprise a purified antigen cross-linked to a carrier protein selected from the group consisting of an albumin protein, an egg white protein or mixture of egg white proteins, and gelatin. In some examples, the present disclosure relates to tissue microarrays (TMAs) comprising at least a first and a second solid antigen/carrier protein gel, wherein the first and second solid antigen/carrier protein gels both comprise a purified antigen cross-linked to a carrier protein selected from the group consisting of an albumin protein, an egg white protein or mixture of egg white proteins, gelatin, and poly-lysine. In some examples, the first solid antigen/carrier protein gel comprises a first purified antigen, and the second solid antigen/carrier protein gel comprises a second purified antigen different from the first purified antigen. In some examples, the first solid antigen/carrier protein gel comprises a first purified antigen at a first concentration, and the second solid antigen/carrier protein gel comprises the first purified antigen at a second concentration different from the first concentration.

[0019a] In one aspect, the present disclosure refers to a tissue microarray (TMA) comprising at least a first and a second solid antigen/carrier protein gel, wherein the first and second solid antigen/carrier protein gels both comprise a purified antigen cross-linked to a carrier protein, wherein the carrier protein consists of a serum albumin protein and the solid gels have been fixed in a fixative comprising formaldehyde.

[0020] Certain examples of the present disclosure relate to methods for immunohistochemical (IHC) staining of an antigen, comprising: providing a solid antigen/carrier protein gel according to any one of the above examples, wherein the solid antigen/carrier protein gel contains the antigen; providing a sample; contacting the solid antigen/carrier protein gel and the sample with a primary antibody that specifically binds the antigen; after contacting the solid antigen/carrier protein gel and the sample with the primary antibody, contacting the solid antigen/carrier protein gel and the sample with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody; detecting a signal of the detectable moiety from the solid antigen/carrier protein gel; and detecting a signal of the detectable moiety from the sample, wherein detection of a signal from the sample as compared to the signal detected from the solid antigen/carrier protein gel indicates the presence of the antigen in the sample. In some examples, the detectable

moiety comprises an enzyme, and wherein detecting the signal of the detectable moiety comprises exposing the detectable moiety to a chromogenic, fluorescent or chemiluminescent substrate of the enzyme and detecting a signal from the substrate upon reaction with the enzyme. In some examples, the detectable moiety comprises a fluorophore, metal particle, metal ion, radioisotope, nucleic acid, electrochemiluminescent reporter, or a "quantum dot" (a solid state, semiconductor or carbon-based fluorescent nanoparticle). In some examples, the sample is a tissue sample.

[0020a] In one aspect, the present disclosure refers to a method for immunohistochemical (IHC) staining of an antigen, comprising:

providing a solid antigen/carrier protein gel as disclosed herein;

providing a sample;

contacting the solid antigen/carrier protein gel and the sample with a primary antibody that specifically binds the antigen;

after contacting the solid antigen/carrier protein gel and the sample with the primary antibody, contacting the solid antigen/carrier protein gel and the sample with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody;

detecting a signal of the detectable moiety from the solid antigen/carrier protein gel; and

detecting a signal of the detectable moiety from the sample, wherein detection of a signal from the sample as compared to the signal detected from the solid antigen/carrier protein gel indicates the presence of the antigen in the sample.

[0021] Certain examples of the present disclosure relate to methods for control immunohistochemical (IHC) staining of an antigen, comprising: providing a first and a second solid antigen/carrier protein gel according to any one of the above examples, wherein the first solid antigen/carrier protein gel contains the antigen at a first concentration, and wherein the second solid antigen/carrier protein gel contains the antigen at a second concentration higher than the first concentration; contacting the first and the second solid antigen/carrier protein gels with a primary antibody that specifically binds the antigen; after contacting the first and the second solid antigen/carrier protein gels with the primary antibody, contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody; detecting a first signal of the detectable moiety from the first solid antigen/carrier protein gel; and detecting a second signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of a second signal greater than the first signal indicates control IHC staining of the antigen. In some examples, the detectable moiety comprises an enzyme, and wherein detecting the signal of the detectable moiety comprises exposing the detectable moiety to a chromogenic, fluorescent or chemiluminescent substrate of the enzyme and detecting a signal from the substrate upon reaction with the enzyme. In some examples, the detectable moiety comprises a fluorophore, metal particle, metal ion, radioisotope, nucleic acid, electrochemiluminescent reporter, or a "quantum dot" (a solid state, semiconductor or carbon-based fluorescent nanoparticle). In some examples, the first concentration is 0nM, and wherein detection of a lack of a first signal indicates control IHC staining of the antigen. In some examples, the methods further comprise: providing a sample; contacting the sample with the primary antibody; after contacting the sample with the primary antibody, contacting the sample with the secondary antibody; detecting a third signal

of the detectable moiety from the sample; and comparing the third signal with the first and the second signals, wherein an amount of the third signal relative to the amounts of the first and the second signals indicates an abundance of the antigen in the sample, relative to the amounts of the antigen in the first and the second solid antigen/carrier protein gels.

[0021a] In one aspect, the present disclosure refers to a method for control immunohistochemical (IHC) staining of an antigen, comprising:

providing a first and a second solid antigen/carrier protein gel, wherein each of the first and the second solid antigen/carrier protein gels is as disclosed herein, wherein the first solid antigen/carrier protein gel contains the antigen at a first concentration, and wherein the second solid antigen/carrier protein gel contains the antigen at a second concentration higher than the first concentration;

contacting the first and the second solid antigen/carrier protein gels with a primary antibody that specifically binds the antigen;

after contacting the first and the second solid antigen/carrier protein gels with the primary antibody, contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody;

detecting a first signal of the detectable moiety from the first solid antigen/carrier protein gel; and

detecting a second signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of a second signal greater than the first signal indicates control IHC staining of the antigen.

[0022] Certain examples of the present disclosure relate to methods for control immunohistochemical (IHC) staining with a secondary antibody, comprising: providing a first and a second solid antigen/carrier protein gel according to any one of the above examples, wherein the first solid antigen/carrier protein gel comprises a first antibody having a first isotype, and wherein the second solid antigen/carrier protein gel comprises a second antibody having a second isotype different from the first isotype; contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the first isotype, wherein a detectable moiety is conjugated to the secondary antibody; detecting a signal of the detectable moiety from the first solid antigen/carrier protein gel; and detecting a lack of the signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of the signal associated with the first solid antigen/carrier protein gel and the lack of the signal associated with the second solid antigen/carrier protein gel indicates control staining with the secondary antibody. In some examples, the detectable moiety comprises an enzyme, and wherein detecting the signal of the detectable moiety comprises exposing the detectable moiety to a chromogenic, fluorescent or chemiluminescent substrate of the enzyme and detecting a signal from the substrate upon reaction with the enzyme. In some examples, the detectable moiety comprises a fluorophore, metal particle, metal ion, radioisotope, nucleic acid, electrochemiluminescent reporter, or a "quantum dot" (a solid state, semiconductor or carbon-based fluorescent nanoparticle).

[0022a] In one aspect, the present disclosure refers to a method for control immunohistochemical (IHC) staining with a secondary antibody, comprising:

providing a first and a second solid antigen/carrier protein gel, wherein each of the first and the second solid antigen/carrier protein gels is as disclosed herein, wherein the first solid antigen/carrier protein gel comprises a first antibody having a first isotype, and wherein the second solid antigen/carrier protein gel comprises a second antibody having a second isotype different from the first isotype;

contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the first isotype, wherein a detectable moiety is conjugated to the secondary antibody;

detecting a signal of the detectable moiety from the first solid antigen/carrier protein gel; and

detecting a lack of the signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of the signal associated with the first solid antigen/carrier protein gel and the lack of the signal associated with the second solid antigen/carrier protein gel indicates control staining with the secondary antibody.

[0023] Certain examples of the present disclosure relate to kits (*e.g.*, for control immunohistochemical (IHC) staining of an antigen) comprising: a first solid gel comprising a purified antigen cross-linked to a carrier protein selected from the group consisting of an albumin protein (*e.g.*, a serum albumin protein), an egg white protein or a mixture of egg white proteins, and gelatin, wherein the purified antigen is present in the first solid gel at a first concentration; and a second solid gel comprising the purified antigen cross-linked to the carrier protein, wherein the purified antigen is present in the second solid gel at a second concentration different from the first concentration. In some examples, the kits comprise: a first solid gel

comprising a purified antigen cross-linked to a carrier protein selected from the group consisting of an albumin protein (*e.g.*, a serum albumin protein), an egg white protein or a mixture of egg white proteins, gelatin, and poly-lysine, wherein the purified antigen is present in the first solid gel at a first concentration; and a second solid gel comprising the purified antigen cross-linked to the carrier protein, wherein the purified antigen is present in the second solid gel at a second concentration different from the first concentration. In some examples, the first and the second solid gels are each sectioned into a thickness of between about 30nm and about 50µm. In some examples, the first and the second solid gels are affixed to one or more solid substrates. In some examples, the kits further comprise: a third solid gel comprising the carrier protein, wherein the third solid gel does not comprise the purified antigen. In some examples, the third solid gel is sectioned into a thickness of between about 30nm and about 50µm. In some examples, the third solid gel is affixed to a solid substrate.

[0023a] In one aspect, the present disclosure refers to a kit for control immunohistochemical (IHC) staining of an antigen, comprising:

(a) a first solid gel comprising a purified antigen cross-linked to a carrier protein, wherein the purified antigen is present in the first solid gel at a first concentration; and

(b) a second solid gel comprising the purified antigen cross-linked to the carrier protein, wherein the purified antigen is present in the second solid gel at a second concentration different from the first concentration,

wherein the carrier protein consists of a serum albumin protein and the solid gels have been fixed in a fixative comprising formaldehyde.

[0023b] In one aspect, the present disclosure refers to a method for generating a solid antigen/carrier protein gel for immunohistochemical (IHC) staining, comprising:

(a) mixing a purified antigen with a liquid solution consisting of a carrier protein and a buffer to produce an antigen/carrier protein liquid solution;

(b) heating the antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel, and

(c) contacting the solid antigen/carrier protein gel with a fixative,

wherein the carrier protein consists of a serum albumin protein.

[0024] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the art. These and other examples of the disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0026] **FIG. 1** shows an exemplary workflow for IHC staining using slides obtained from tissue samples.

[0027] **FIG. 2A** shows a bovine serum albumin (BSA) gel created in, then removed from, a 1.5mL microcentrifuge tube.

[0028] **FIGS. 2B & 2C** show different views of sections of a BSA gel created in, then removed from, a 1.5mL microcentrifuge tube.

[0029] **FIG. 2D** shows a sliced gel portion prepared for dehydration and paraffin processing.

CLAIMS

What is claimed is:

1. A method for generating a solid antigen/carrier protein gel for immunohistochemical (IHC) staining, the method comprising:

(a) mixing a purified antigen with a liquid solution comprising a carrier protein to produce an antigen/carrier protein liquid solution; and

(b) heating the antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel,

wherein the carrier protein consists of a serum albumin protein and the antigen/carrier protein liquid solution further comprises formaldehyde.

2. The method of claim 1, further comprising, after (b): dehydrating the solid antigen/carrier protein gel and embedding the dehydrated solid antigen/carrier protein gel in a paraffin block.

3. The method of claim 1, further comprising, after (b): incubating the solid antigen/carrier protein gel in a liquid embedding medium and freezing the solid antigen/carrier protein gel in the embedding medium.

4. The method of claim 1, further comprising, after (b): embedding the solid antigen/carrier protein gel in a plastic resin.

5. The method of claim 1, wherein the antigen/carrier protein liquid solution comprises formaldehyde at a final concentration of at least about 1%.

6. The method of claim 1, further comprising:

after (b), dehydrating the solid antigen/carrier protein gel;

embedding the dehydrated solid antigen/carrier protein gel in a paraffin block;

sectioning the paraffin block with the embedded antigen/carrier protein gel into one or more sections having a thickness of between about 30nm and about 50µm;

subjecting the one or more sections of the embedded solid antigen/carrier protein gel to antigen retrieval; and

after antigen retrieval, blocking the one or more sections of the embedded solid antigen/carrier protein gel.

7. The method of claim 1, further comprising:

after (b), incubating the solid antigen/carrier protein gel in a liquid embedding medium;

freezing the solid antigen/carrier protein gel in the embedding medium;

sectioning the frozen antigen/carrier protein gel into one or more sections having a thickness of between about 30nm and about 50µm; and

blocking the one or more sections of the frozen antigen/carrier protein gel.

8. The method of claim 1, wherein the antigen is a polypeptide antigen.

9. The method of claim 8, wherein the antigen comprises an N-terminal tyrosine, a C-terminal cysteine, or both.

10. The method of claim 9, further comprising, prior to (b), cross-linking the antigen with the carrier protein using a cysteine-reactive reagent.

11. The method of claim 1, wherein the serum albumin protein is bovine serum albumin.

12. The method of claim 1, wherein the antigen/carrier protein liquid solution produced in (a) comprises the carrier protein at a concentration of greater than or equal to 2% (w/v) and less than or equal to 25% (w/v).

13. A solid antigen/carrier protein gel for immunohistochemical (IHC) staining comprising a purified antigen cross-linked to a carrier protein, wherein the carrier protein consists of a serum albumin protein and the solid gel has been fixed in a fixative comprising formaldehyde.

14. The solid gel of claim 13, wherein the solid gel is frozen in an embedding medium.

15. The solid gel of claim 13, wherein the solid gel is embedded in paraffin.

16. The solid gel of claim 13, wherein the solid gel is embedded in a plastic resin.

17. The solid gel of claim 13, wherein the solid gel is affixed to a solid substrate.

18. A tissue microarray (TMA) comprising at least a first and a second solid antigen/carrier protein gel, wherein the first and second solid antigen/carrier protein gels both comprise a purified antigen cross-linked to a carrier protein, wherein the carrier protein consists of a serum albumin protein and the solid gels have been fixed in a fixative comprising formaldehyde.

19. A method for immunohistochemical (IHC) staining of an antigen, comprising:

providing a solid antigen/carrier protein gel according to claim 13;

providing a sample;

contacting the solid antigen/carrier protein gel and the sample with a primary antibody that specifically binds the antigen;

after contacting the solid antigen/carrier protein gel and the sample with the primary antibody, contacting the solid antigen/carrier protein gel and the sample with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody;

detecting a signal of the detectable moiety from the solid antigen/carrier protein gel; and

detecting a signal of the detectable moiety from the sample, wherein detection of a signal from the sample as compared to the signal detected from the solid antigen/carrier protein gel indicates the presence of the antigen in the sample.

20. A method for control immunohistochemical (IHC) staining of an antigen, comprising:

providing a first and a second solid antigen/carrier protein gel, wherein each of the first and the second solid antigen/carrier protein gels is according to claim 13, wherein the first solid antigen/carrier protein gel contains the antigen at a first concentration, and wherein the second

solid antigen/carrier protein gel contains the antigen at a second concentration higher than the first concentration;

contacting the first and the second solid antigen/carrier protein gels with a primary antibody that specifically binds the antigen;

after contacting the first and the second solid antigen/carrier protein gels with the primary antibody, contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the primary antibody, wherein a detectable moiety is conjugated to the secondary antibody;

detecting a first signal of the detectable moiety from the first solid antigen/carrier protein gel; and

detecting a second signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of a second signal greater than the first signal indicates control IHC staining of the antigen.

21. A method for control immunohistochemical (IHC) staining with a secondary antibody, comprising:

providing a first and a second solid antigen/carrier protein gel, wherein each of the first and the second solid antigen/carrier protein gels is according to claim 13, wherein the first solid antigen/carrier protein gel comprises a first antibody having a first isotype, and wherein the second solid antigen/carrier protein gel comprises a second antibody having a second isotype different from the first isotype;

contacting the first and the second solid antigen/carrier protein gels with a secondary antibody that specifically binds the first isotype, wherein a detectable moiety is conjugated to the secondary antibody;

detecting a signal of the detectable moiety from the first solid antigen/carrier protein gel; and

detecting a lack of the signal of the detectable moiety from the second solid antigen/carrier protein gel, wherein detection of the signal associated with the first solid antigen/carrier protein gel and the lack of the signal associated with the second solid antigen/carrier protein gel indicates control staining with the secondary antibody.

22. A kit for control immunohistochemical (IHC) staining of an antigen, comprising:

(a) a first solid gel comprising a purified antigen cross-linked to a carrier protein, wherein the purified antigen is present in the first solid gel at a first concentration; and

(b) a second solid gel comprising the purified antigen cross-linked to the carrier protein, wherein the purified antigen is present in the second solid gel at a second concentration different from the first concentration,

wherein the carrier protein consists of a serum albumin protein and the solid gels have been fixed in a fixative comprising formaldehyde.

23. A method for generating a solid antigen/carrier protein gel for immunohistochemical (IHC) staining, comprising:

(a) mixing a purified antigen with a liquid solution consisting of a carrier protein and a buffer to produce an antigen/carrier protein liquid solution;

(b) heating the antigen/carrier protein liquid solution to form the solid antigen/carrier protein gel, and

(c) contacting the solid antigen/carrier protein gel with a fixative,

wherein the carrier protein consists of a serum albumin protein.

24. The method of claim 23, wherein the fixative comprises formaldehyde.

25. The method of claim 23, wherein the fixative comprises glutaraldehyde, Davidson's fixative, Bouin's fixative, ½ strength Karnovski's fixative, or a zinc salt.

26. The method of claim 23, further comprising, after (c), sectioning the solid antigen/carrier protein gel into one or more solid antigen/carrier protein gel sections having a thickness of between about 30nm and about 50µm.

27. The method of claim 23, wherein the serum albumin protein is bovine serum albumin.