METHOD AND APPARATUS FOR ANTIGEN RETRIEVAL PROCESS

BACKGROUND

[0001] Diagnostic cell imaging uses methods whereby molecules produced by cells or tissues can be specifically localized to those cells or tissues. This provides a researcher with information as to the sites of production or activity of those given molecules. For the specific localization of proteins in routine pathology, a procedure known as immunohistochemistry (IHC) is routinely utilized. In IHC an antibody for a specific antigen is applied to a fixed tissue sample that recognizes a known specific molecule as a first step. This antibody is then detected with the use of a secondary antibody that has been chemically coupled with an enzyme, such as horseradish peroxidase. After incubation with a chromogenic substrate such as di-amino-benzidine (DAB), a colored deposit is produced at the site of the secondary antibody that has bound to the primary antibody at the specific site of the protein of interest. This procedure is currently utilized for over 200 antibodies in clinical pathology labs and many more in the research environment.

[0002] The nature of tissue processing requires that the samples be "fixed" prior to embedding in paraffin and micro-sectioning on a microtome to produce tissue sections suitable for immunostaining. During this process, proteins are preserved using a formaldehyde treatment that produces chemical cross-linking which preserves the cellular features of the tissue. Formaldehyde preserves or fixes tissue or cells predominantly by cross-linking primary amine groups in proteins with other nearby nitrogen atoms in protein or DNA through a -CH2- linkage. The process of tissue fixation however, frequently masks antigens on specific proteins for which detection is desirable for diagnostic and prognostic purposes. Typically procedures are optimized for detection of individual target molecules, and when needed, serial sections are processed in a different manner for the detection of additional targets molecules. With advances in the ability to detect multiple antigens in a single sample, there needs to be a uniform tissue processing that is compatible with the detection of multiple proteins.

BRIEF DESCRIPTION

[0003] In a first aspect, there is ~~invention~~ provided a method of antigen retrieval of a formaldehyde- fixed tissue sample comprising the steps of: incubating the formaldehyde-fixed tissue sample in a first antigen retrieval solution at a temperature of greater than 90 $^{\circ}$ C; transferring the formaldehyde-fixed tissue

sample to a second antigen retrieval solution;, and incubating the formaldehyde-fixed tissue sample in the second antigen retrieval solution at a temperature of greater than 90°C; wherein the first antigen retrieval solution comprises a buffer solution having a pH range of between about 5 and about 7 and the second antigen retrieval solution comprises a buffer solution having a pH range of between about 7.5 and about 11; or the first antigen retrieval solution comprises a buffer solution having a pH range of between about 7.5 and about 11 and the second antigen retrieval solution comprises a buffer solution having a pH range of between about 5 and about 7; and wherein said steps of using the first and second antigen retrieval solutions enhances antigen retrieval in the tissue relative to using either solution alone.

[0004] In a second aspect, there is ~~invention~~ provided a kit for retrieving antigens in a formaldehyde-fixed tissue sample, comprising:, a first antigen retrieval solution that retrieves at least a portion of unretrieved antigens in the sample;, and a second antigen retrieval solution that retrieves at least some of another portion of unretrieved antigens in the sample; wherein the first antigen retrieval solution is a buffer solution having a pH range between about 5 and about 7 and the second antigen retrieval solution is a buffer solution having a pH range of between about 7.5 and about 11; and wherein the first antigen retrieval solution comprises citric acid, potassium dihydrogen phosphate, boric acid, diethyl barbituric acid, piperazine-N, N'-bis (2-ethanesulfonic acid), dimethylarsinic acid, 2-(N-morpholino)ethanesulfonic acid, or a combination thereof; and wherein the second antigen retrieval solution comprises tris(hydroxymethyl)methylamine (TRIS) , 2-(N-morpholino)ethanesulfonic acid (TAPS), N,N-bis(2-hydroxyethyl)glycine(Bicine), N-tris(hydroxymethyl)methylglycine (Tricine), 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid (TES), or a combination thereof.

[0005] In one embodiment~~a third aspect~~, the disclosure~~invention~~ provides a sample handling device for carrying out the an antigen retrieval method comprising a sample handling subsystem, a reagent dispensing subsystem, and a signal detection subsystem.

DRAWINGS

[0006] These and other features, aspects, and advantages of the present invention will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings.

[0007] FIG. 1 is a representative sample handling device for contacting a formaldehyde fixed tissue sample with an antigen retrieval solutions.

[0008] FIG. 2 is a monochromatic micrograph (at 20X or 63X magnification) showing enhanced staining using a two-step procedure compared to a one step procedure.

[0009] FIG. 3 shows monochromatic micrographs (at 20X magnification) showing enhanced staining using a two step procedure in a multiplex analysis FISH of BrCA tumor.

[0010] FIG. 4 is a bar chart of quantitative analysis results comparing a manual two-step antigen retrieval method to automated methods.

[0011] FIG. 5 shows monochromatic micrographs (at 20X magnification) of the bleaching effect on antigen-S6 staining on sample prepared by either the manual two-step antigen retrieval method or one of two automated antigen retrieval processes.