

The present invention provides an improved, robust and fast ISH method for detection of non-coding RNAs in FFPE-samples. The method avoid use of the teratogenic formamide while providing even better ISH-results than obtained with standard formamide comprising hybridization buffers. Furthermore the method has the advantage that it can be used for
5 quantification of small non-coding RNAs by ISH in archival FFPE-samples.

SUMMARY OF THE INVENTION:

Prior to the present invention, the present inventors believed that foramide was an
10 indispensable component of a successful ISH hybridization mix.

However, the present inventors, surprisingly, observed that formamide may be substituted by certain chaotropic substances such as, for example, urea and guanidine hydrochloride to obtain even better ISH-results than obtained with standard formamide comprising
15 hybridization buffers.

Thus, in a first aspect, the invention pertains to a method for detection of nucleic acids by in situ hybridization of an fixed cellular specimen comprising a hybridization step which is performed in a formamide-free, hybridization buffer that comprise a chaotropic component
20 selected from the group of urea, salts of guanidinium or guanidine and a mixture of two or more members of the group, wherein the nucleic acids are small, non-coding RNA molecules.

Also disclosed is a method for detection of nucleic acid molecules comprising a contiguous
25 a nucleotide sequence such as mRNA by in situ hybridisation in fixed cellular specimens comprising of a hybridization step which is performed in a formamide-free, hybridization buffer that comprises a chaotropic component selected from the group of urea, salts of guanidinium or guanidine and a mixture of two or more members of the group.

30 In one ~~preferred embodiment~~ example, the invention pertains to a method for detection of small, non-coding RNAs by in situ hybridisation in fixed cellular specimens comprising of a hybridization step which is performed in a formamide-free, hybridization buffer that comprises a chaotropic component selected from the group of urea, salts of guanidinium or guanidine and a mixture of two or more members of the group.

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In a further ~~preferred embodiment~~ example, the improved, formamide-free, hybridization buffer is used of detecting nucleic acid molecules in standard formalin-fixed and paraffin-embedded (FFPE) tissue sections or in frozen (cryostat) tissue sections.

5 In a second aspect the invention provide an improved, formamide-free, hybridization buffer for the use of detecting small non-coding RNAs in standard formalin-fixed and paraffin-embedded (FFPE) tissue sections with LNA-probes by in situ hybridization, the buffer comprise:

- 10 - a buffer comprising a hybrid stabilizing agent selected from the group of salts of mono- and di-valent cations,
- 0.5 to 5 M of a chaotropic component selected from the group comprised of urea and salts of guanidinium (or guanidine) or a mixture of two or more members of the group,
- Denhardt's solution in an amount of 0 - 2 x, and
- a carrier RNA, such as yeast t-RNA, in an amount of 0- 0.5 mg/ml.

15 Also disclosed is an improved, formamide-free, hybridization buffer for the use of detecting nucleic acid molecules comprising a contiguous a nucleotide sequence such as mRNA, rRNA or small non-coding RNAs in tissue sections with probes,

in particularly LNA-probes, by in situ hybridization, the buffer comprise 0.5 to 5 M of a chaotropic component selected from the group comprised of urea and salts of guanidinium (or guanidine) or a mixture of two or more members of the group.

5 | In one example, there is provided~~a preferred embodiment of the invention~~ an improved, formamide-free, hybridization buffer for detecting nucleic acid molecules in standard formalin-fixed and paraffin-embedded (FFPE) tissue sections ~~is provided~~.

According to the present invention, there is also provided a kit for detection of at least one
10 small non-coding RNAs in standard formalin-fixed and paraffin-embedded (FFPE) tissue by in situ hybridization, said kit comprise the improved, formamide-free, hybridization buffer and least one LNA-probe optimized for the specific detection of said one small non-coding RNA.

15 A further advantage of the herein disclosed method is its robustness and low variance allowing it to be used for semi-quantitative in situ hybridization allowing for miRNA-associated diagnostics such as for a method of estimating/evaluating disease-free survival in stage II colon cancer comprising:

- a) determining the relative level of miR-21 in at least one representative tissue section
20 | from a stage II colon cancer of said patient determined by the method of claim 57,
- b) comparing the level of miR-21 in the patient to a set of relative levels of miR-21
| obtained by the method of claim 57 from a reference panel of stage II colon cancer samples obtained from a reference panel of patients with known disease history,
- c) grouping the reference panel in tertiles according to the relative level of miR-21
25 | determined by the method of claim 57; and
- d) taking the miR-21 level of said at least one representative tissue section from a stage II colon tumor from said patient that falls within the miR-21 level of the upper (high expressing) tertile as indicative of an increased likelihood of short disease-free survival, and taking a miR-21 level of said at least one representative tissue
30 | section from a stage II colon tumor from said patient that falls within the miR-21 level of the lower (low expressing) tertile as indicative of an increased likelihood of long disease-free survival.

DEFINITIONS

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Prior to a discussion of the detailed embodiments of the invention is provided a definition of specific terms related to the main aspects of the invention.