DATA SHEET-V4 HYBRIDISATION MIXTURES

Product **Oligonucleotide *in situ* hybridisation mixture**
DNA *in situ* hybridisation mixture
Code **R021R.0000, R024R.0000**

Technical specifications

Cat. No.	Description	Contents	Format	Appearance	pH
R021R.0000	Oligonucleotide *in situ* hybridisation mixture (HYB MIX OLIGO)	10 mL hybridisation solution; contains dextrane sulphate	Ready to use	Clear liquid; viscous	7.0±0.5
R024R.0000	DNA *in situ* hybridisation mixture (HYB MIX DNA)	10 mL hybridisation solution contains SSC, dextrane sulphate, fish sperm and formamid	Ready to use	Clear liquid; viscous	8.0±0.5

Application : - diluent for probes: dilute labelled probes to a final concentration of 1-5 ng/ul and determine optimal concentration using the equation: Tm = 49.82 + 0.41 (%G+C) – 600/n (n = length of probe in bp); the optimal hybridisation temperature is 25 °C below Tm; for use in *in situ* hybridisation (ISH)
- pre-hybridisation step for reducing background staining

Storage : refrigerated (2-8 °C) protected from light; do not freeze

Stability : until expiry date printed on label

Precautions : - homogenise solutions before use
- harmful, avoid contact with eyes and skin; do not swallow

Related products

Please contact your local supplier for further information.

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Limitations of Procedure

Product Hybridisation mixtures

- The REMBRANDT® *in situ* hybridisation mixtures are solely applicable as a pre-hybridisation step and/or as a probe diluent in ISH.
- Appropriate medical decisions are only possible if the medical traceability is ensured. The product is intended for professional use as an aid in the diagnosis by *in situ* hybridisation.
- Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. Sections should be cut at 4 um thickness, glued to the glass slides with a bio-adhesive (e.g. organosilane), dried at room temperature, subsequently dried at 37 °C overnight, complete deparaffinisation in xylene and alcohol series and air dried. Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.
- Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, incubation temperatures, all other reagents (i.e. deparaffinisation reagents, proteolytic treatment, probes etc.) used in the procedure and interpretation of results.
- The performance of the ISH procedure is also affected by the sensitivity of the method and the DNA or RNA target load; in case the limit of the sensitivity is reached or when the target DNA or RNA load is too low, a false negative reaction may be the result.
- The clinical interpretation of the results should not be established on the basis of a single test result. Moreover, diagnosis should also take the clinical history, symptoms, as well as morphological data into consideration. Negative results therefore do not rule out any possibility of a positive specimen.
- The REMBRANDT® test results are not to be relied on in case the sampling, sampling method, quality, sample preparation, other reagents used, controls and procedure followed is not optimal.
- Therapeutic considerations based on the result of this test alone should not been taken. Positive results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.
- The medical profession should be aware of risks and factors influencing the intensity, the absence or presence of probe signals which can not be foreseen when applying this test.
- The user should carefully consider the risk and use of sample material for this test in case the sample material does not contain sufficient or representative test material.
- Laboratory personnel performing the test should be knowledgeable and be able to interpret the test results.

Interpretation of the results

First, check the negative and positive controls that have been incubated with the test slides simultaneously:

- The negative control should be really negative, i.e. not show any localised colour precipitations. If the negative control could be interpreted as being positive, discard the results since no conclusions can be drawn.
- The positive control should show colour precipitations in conformity with the localisation of the target DNA or RNA. The colour should show the proper shade and must be clearly visible in the preferential cell/ tissue type and correspond to the target localisation.

In the test slides, start under low power magnification and focus on localisation and colour to see whether:

- The positivity (colour precipitation) observed is localised in the cell type preferred by the target.
- The colour has the right shade (no endogenous or formalin pigment).

Use high power magnification to see whether:

- The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern.

Product in combination with other devices

The REMBRANDT® *in situ* hybridisation mixtures are intended for stand-alone usage. The in vitro diagnostic is intended to be used in combination with standard formalin fixed, paraffin embedded tissue blocks, standard tissue freezing, tissue sectioning (microtome), standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), other needed reagents (but not supplied with this reagent) for ISH and a microscope. The combination has been tested and validated. Since the standard formalin fixed, paraffin embedded tissue blocks, standard tissue freezing, tissue sectioning (microtome), standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other needed reagents such as but not limited to proteolytic reagents, labelled probes, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the results'* and are also depending on the target; since the *in situ* hybridisation mixtures can be used for all ISH applications and are also depending on the target load, the validation criteria may vary.

References

1. Keller GH, DNA probes, Stockton Press, ISBN 0-935859-63-2 (1989).

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