

FlexmiR™ Reagent Pak

For Research Use Only. Not for use in diagnostic procedures.

Summary and Intended Use

The FlexmiR Reagent Pak is designed for use with the FlexmiR Select assay and FlexmiR microRNA panels for microRNA (miRNA) expression analysis. The FlexmiR Reagent Pak includes reagents for hybridization of labeled total RNA to probe-coupled microspheres, post-hybridization washing and addition of fluorescent reporter molecule.

The FlexmiR microRNA assay system measures the expression of your custom selected microRNA sequences by combining xMAP® technology and Locked Nucleic Acid (LNA™) technology. The integration of these technologies allows precise detection of miRNAs without prior need for sample RNA size fractionation or amplification. Discrimination between closely related miRNA family members is achieved by the use of LNA-enhanced capture probes complementary to the miRNA targets. The capture probes have been Tm-normalized to hybridize optimally under the conditions described in this protocol by varying the LNA content and the length of the capture probes.

The FlexmiR microRNA assay system flow allows researchers to (i) biotinylate the 3' ends of total RNA, (ii) hybridize the labeled RNA to Tm-normalized LNA capture probes that have been coupled to fluorescently dyed xMAP microspheres, (iii) tag the captured biotinylated miRNAs with streptavidin-phycoerythrin (SAPE), and (iv) analyze the samples on a Luminex analyzer capable of both identifying the fluorescent microsphere and measuring the SAPE intensity of the labeled microRNA target captured by the microsphere.

Reagent Pak Contents

The FlexmiR Reagent Pak (part number FMIR-PAK-100) consists of 4 components and provides sufficient reagents for 100 hybridization reactions.

Kit Component	Amount supplied
Hybridization Solution	1.6 mL
Wash Solution	50 mL
Reporter Molecule (SA-PE)	50 µL
Nuclease-Free Water	3.6 mL

Safety Precautions

Although this product does not contain hazardous or carcinogenic components at toxic levels, it may be toxic if swallowed. If product comes in contact with skin, remove contaminated clothing and wash with soap and water. In case of accidents or if you feel unwell seek medical advice immediately and show this product label to your medical provider. Material Safety Data Sheet available upon request. Use standard lab practices for handling and storage of RNA to prevent RNase contamination or degradation.

Limitations

The FlexmiR Reagent Pak is designed explicitly for use with the FlexmiR Select assay and FlexmiR microRNA panels for microRNA (miRNA) expression analysis. These reagents have not been tested for use with other microRNA detection systems.

Product Storage

The FlexmiR MicroRNA Reagent Pak ships at ambient temperature. Upon receipt, store the reagents at 2-8 °C. Protect the Reporter Molecule from light.

Protocol

Detailed instructions for using the FlexmiR Reagent Pak are provided in the assay-specific instruction manuals, which are provided with the FlexmiR Select assay, FlexmiR MicroRNA Human Panel and FlexmiR MicroRNA Mouse/Rat Extension Panel. Alternatively, these instruction manuals are available from the Luminex website: www.luminexcorp.com/microRNA/download.

Note: Prior to performing the post-hybridization wash steps, aliquot and preheat an appropriate volume (min. 200 µL per well) of Wash Solution to 60° C. When making the wash solution aliquots, make separate aliquots for each of the 2 wash steps. This ensures that each aliquot of wash solution is kept warm until used in the protocol. Keep remaining Wash Solution at room temperature.

Note: Prior to using the Reporter Molecule within an assay, prepare fresh reporter solution by diluting the Reporter Molecule in room temperature Wash Solution to 1:300 dilution in a nuclease-free container. Protect this solution from light. Plan to use 75 µL of reporter solution for each reaction.

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