

USB® miRtect-IT™ miRNA Labeling & Detection Kit

- **Fast**—capture and label miRNA in just over 2 hours
- **Sensitive**—detect miRNA in as little as 50 ng or less of total RNA
- **Specific**—measure mature miRNA without an amplification step
- **Quantitative**—linear detection range of 0.2 -20 femtomoles
- **Simple**—one labeling reaction for detection of any miRNA of interest

The miRtect-IT miRNA Labeling and Detection Kit is based on a novel method for the labeling and detection of mature miRNA and small RNAs from total RNA by splinted-ligation technology^(1,2). The splinted-ligation technology is a nucleic acid hybridization assay that uses a miRNA-specific Bridge Oligonucleotide to form base pairs with the miRNA and a Detection Oligonucleotide. The captured miRNA is subsequently ligated to the Detection Oligonucleotide with T4 DNA Ligase (Figure 1).

By joining the miRNA to the radiolabeled Detection Oligonucleotide, the assay directly labels miRNAs and avoids the inherent difficulty of detecting small RNAs of 20-32 nucleotides in Northern blots. Quantitative measurement of miRNAs in total RNA samples can be completed within 6 hours.

miRtect-IT miRNA Labeling and Detection Kit

A highly sensitive, novel method that directly labels miRNAs, and avoids the inherent difficulty of detecting small RNAs with labor intensive Northern Blots.

Sensitivity

The miRNA detected by miRtect-IT is quantitative and shows correlation to Northern blot data—with a 50-fold better detection sensitivity (Figure 2).

Specificity and versatility

The miRtect-IT assay specificity is shown by measuring endogenous miRNAs from 6 different human miRNAs in 4 different tissues and 1 cell culture (Figure 3). The observed miRNA expression is in agreement with published studies that have characterized tissue-specific miRNA expression. In addition, the miRtect-IT assay can be used for detection of other regulatory small RNAs such as PIWI-associated small RNAs (piRNAs) (Figure 4).

Bridge oligonucleotide design

The Bridge Oligonucleotide is a DNA oligonucleotide complementary to both the Detection Oligo and a specific miRNA at its 5' and 3' ends, respectively (Figure 5). Every Bridge Oligonucleotide has the same 14 nucleotide sequence at the 5' end, which allows

a single labeling reaction of the Detection Oligo for detection of any miRNA of interest.

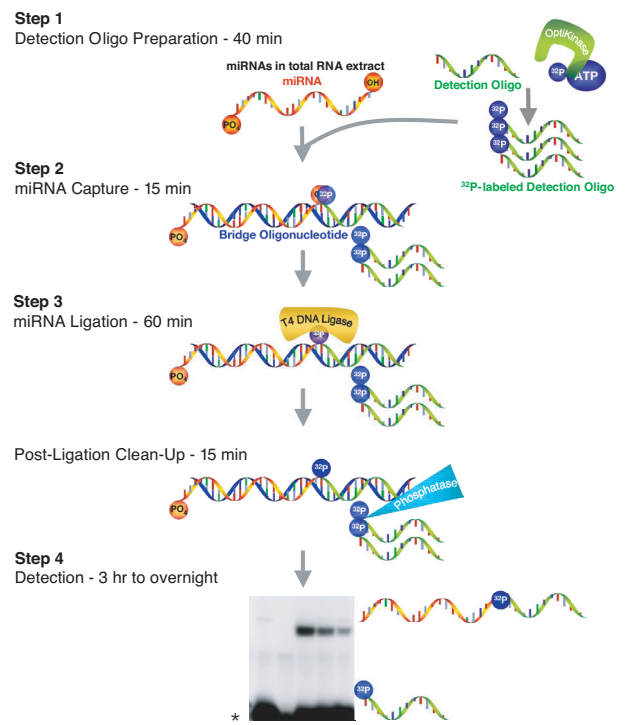
The Bridge Oligonucleotide requires a standard desalted purification after synthesis. Further purification by HPLC or denatured PAGE is usually not necessary. See usb.affymetrix.com for more information on design and preparation of the Bridge Oligonucleotide.

Important: A Bridge Oligonucleotide must be obtained separately. In addition, the assay requires [γ -³²P]-ATP (6000 Ci/mmol, 150 mCi/ml).

Kit components:

Detection Oligo, OptiKinase™, 10X OptiKinase Reaction Buffer, Clean-Up Columns, 10X Capture Buffer, 3X Ligate-IT™ Premix, Positive Control, Clean-Up Mix, Gel Loading Dye, RNase-Free Water

Fig. 1. miRtect-IT protocol



miRtect-IT miRNA Labeling and Detection Kit is a 4-step procedure for direct labeling of miRNA from nanogram to microgram quantities of total RNA.

*Residual radiolabeled 14 nucleotide Detection Oligo will be present due to incomplete removal of the 5' end-³²P labeled Detection Oligo.

References:

- Moore, M. J. and Query, C. C. (2000) *Methods Enzymol.* **317**, 109-123.
- Maroney, P. A., Chamnongpol, S., Souret, F. and Nilsen, T. W. (2007), *RNA*, in press.

miRtect-IT miRNA Labeling and Detection Kit

Product code	Pack size
76400	100 assays

Fig. 2. Quantitative detection of miR-21

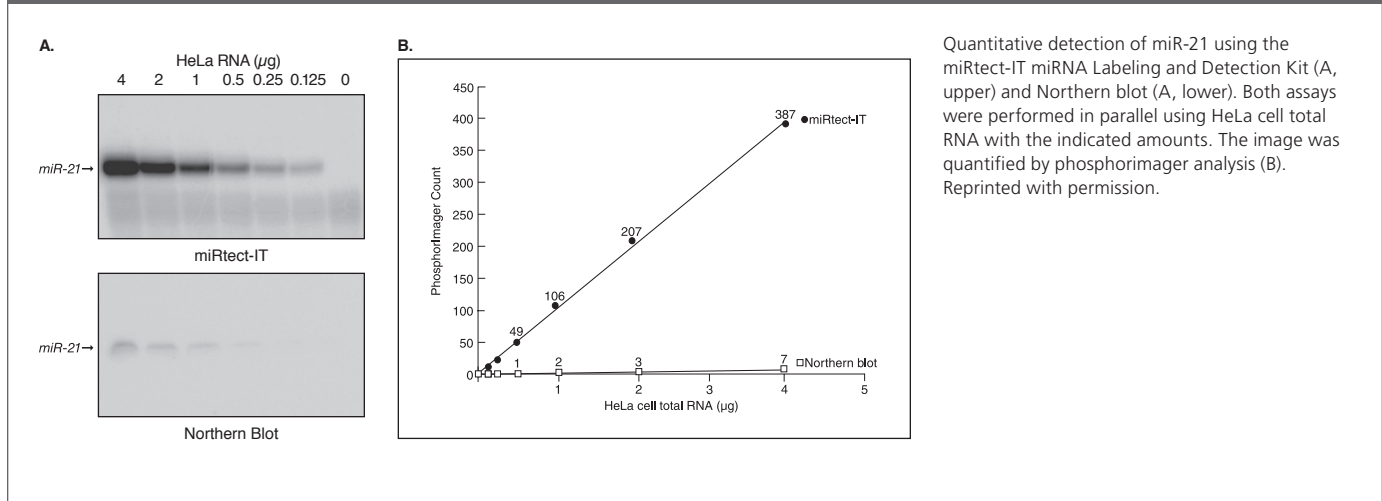


Fig. 3. miRNA detection

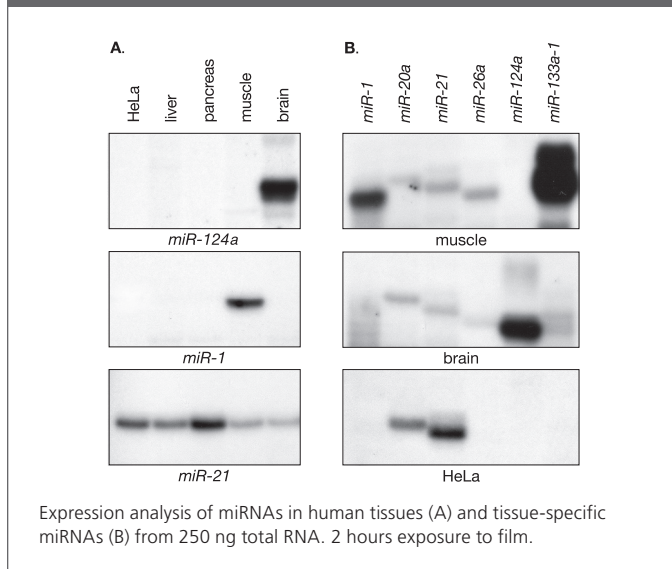


Fig. 4. Small RNA detection

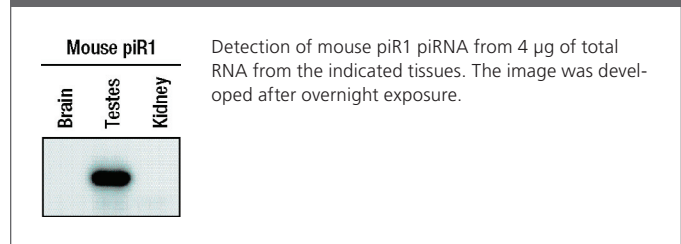


Fig. 5. Bridge oligonucleotide sequence design



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Affymetrix, Inc.
USB® Products
 26111 Miles Road
 Cleveland Ohio 44128
 Tel: 800-321-9322 | 216-765-5000
 Fax: 800-535-0898 | 216-464-5075
 USBcustomerserv@affymetrix.com
usb.affymetrix.com

Affymetrix UK Ltd.
USB® Products
 Voyager, Mercury Park,
 Wycombe Lane, Wooburn Green,
 High Wycombe HP10 0HH, United Kingdom
 Tel: +44 (0)1628 55 2600
 Fax: +44 (0)1628 55 2675
 USBcustomerserveurope@affymetrix.com

Affymetrix Pte Ltd
USB® Products
 7 Gul Circle, #2M-01
 Keppel Logistics Building
 Singapore 629563
 Tel: +65 63957301
 Fax: +65 63957300
 USBcustomerserv@affymetrix.com