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For life science research only. Not for use in diagnostic procedures.

RNA molecular weight marker I, digoxigenin-labeled (0.3–6.9 kb)

Cat. No. 11 526 529 910 4 µg (200 µl)

RNA molecular weight marker II, digoxigenin-labeled (1.5–6.9 kb)

Cat. No. 11 526 537 910 2 µg (200 µl)

RNA molecular weight marker III, digoxigenin-labeled (0.3–1.5 kb)

Cat. No. 11 373 099 910 2 µg (200 µl)

Version 11

Content version: August 2007

Store at −15 to −25° C

1. Product overview

Contents

Product	Conc.	Size range
RNA Molecular Weight Marker I, DIG-labeled	20 ng/µl	0.3-6.9 kb 9 fragments: 310, 438, 575, 1049, 1517, 1821, 2661, 4742 and 6948 bases
RNA Molecular Weight Marker II, DIG-labeled	10 ng/µl	1.5-6.9 kb 5 fragments: 1517, 1821, 2661, 4742 and 6948 bases
RNA Molecular Weight Marker III, DIG-labeled	10 ng/µl	0.3-1.5 kb 5 fragments: 310, 438, 575, 1049 and 1517 bases

Product description

The fragments are prepared by *in vitro* transcription of linearized plasmids with SP6 or T7 RNA polymerase in separate reactions. The transcripts are then combined at a ratio that gives bands of uniform intensity when separated by gel electrophoresis.

The transcripts are labeled in a photodigoxigenin reaction so that a digoxigenin moiety is present every 200th to 300th nucleotide.

Application

For use as size standards in Northern blot analysis when the DIG System for Nucleic Acid Labeling and Detection is used.

The RNA standard, digoxigenin-labeled can be detected simultaneously with hybridized digoxigenin-labeled probes during the immunological digoxigenin detection reaction, allowing a length determination of the non radioactively detected RNA on the blot. Either chemiluminescent detection (*e.g.*, DIG Luminescent Detection Kit*) or colorimetric (*e.g.*, DIG Nucleic Acid Detection Kit*) may be used.

Storage/Stability

The unopened vial is stable at -15 to -25°C until the expiration date printed on the label.

Note: During use, the RNA molecular weight markers should be kept on ice.

** available from Roche Applied Science*

Handling precautions

Extreme care must be taken during handling of RNA Molecular Weight Markers to prevent RNase contamination. **All reagents must be RNase-free.** We therefore recommend:

- wearing gloves for all manipulations
- thawing and keeping RNA markers on ice during all manipulations
- preparing appropriate aliquots of the marker
- using baked glassware
- wiping all surfaces with RNase-Away
- pretreating solutions with DEPC or DMPC and autoclave
- clean gel chambers, combs etc. with DEPC before use
- the use of sterile plastic material, *e.g.*, pipette tips, reaction tubes.

Note: We recommend working according to the protocols listed below.

Quality control

The RNA Molecular Weight Markers, Digoxigenin-labeled exhibit distinct bands of the correct size after denaturing agarose gel electrophoresis, Northern transfer and immunological detection according to the protocol described below.

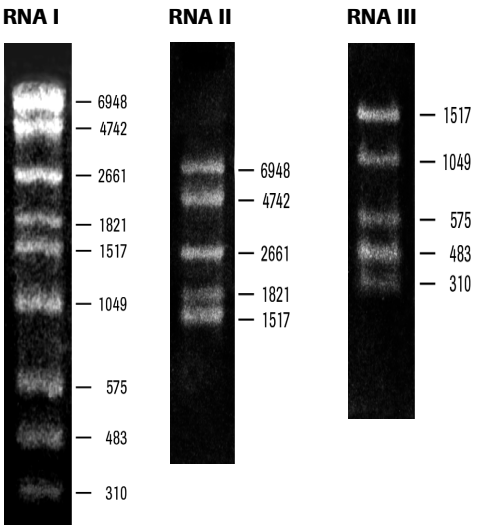
Sensitivity

The table below give the sensitivities to be expected when either the colorimetric (BCIP/NBT*) or chemiluminescent (CSPD*) digoxigenin immunoassays are carried out. The sensitivity depends on the length of time the color signal is allowed to develop, or on the length of exposure to X-ray film or imaging instrument, when the chemiluminescent protocol is used.

Assay	Chemi-luminescent			Color		
Exposure time:	**5 min**	**15 min**	**30 min**	**30 min**	**2 h**	**16 h**
RNA MWM I						
20 ng	-	-	+	-	-	+
40 ng	-	+	++	-	+	++
100 ng	+	++	+++	+	++	+++
RNA MWM II						
10 ng	-	-	+	-	-	+
20 ng	-	+	++	-	+	++
50 ng	+	++	+++	+	++	+++
RNA MWM III						
10 ng	-	+	++	-	-	+
20 ng	+	++	+++	-	+	++
50 ng	++	+++	+++	+	++	+++

+: all bands visible; the number of "+" corresponds to the intensity of the bands.

Characteristic bands of the RNA molecular weight markers, DIG-labeled, after gel electrophoresis



Concentration of agarose gel: 0.9%

Loaded amounts:
RNA I: 40 ng ; RNA II: 20 ng ; RNA III: 20 ng

2. Protocols and required material

2.1 Formaldehyde gels, transfer and fixation.

Formaldehyde gels

- 0.8%–1% agarose gel in 1 × MOPS and 2% formaldehyde (v/v) is recommended for RNA Molecular Weight Markers I and II. Higher agarose concentrations (over 1 %) will reduce the transfer rate (and subsequent visibility) of the 4.7 kb and the 6.9 kb fragments.
- 1%–2% agarose gel in 1× MOPS and 2% formaldehyde (v/v) is recommended for RNA Molecular Weight Marker III.
- For best resolution and separation of the RNA molecular weight markers, we recommend to use 2% formaldehyde agarose gels.
- For electrophoresis the gel should be prepared fresh and poured as thin as possible. Gels lacking ethidium bromide are preferred, because ethidium bromide may cause uneven background problems if the gel is not run long enough. Ethidium bromide staining and destaining of gels should be done to ensure that the loaded RNA is intact.

Recommended RNA marker loading amounts

Transfer of 40 ng–100 ng of the labeled RNA Molecular Weight Marker I per lane or 20 ng–50 ng of RNA Molecular Weight Marker II or III per lane, depending on the reaction time in the detection step, gives a clearly visible banding pattern.

Note: Taking the lane (slot) size of the agarose gel into account we recommend to load 100 ng (for RNA marker I) and 50 ng (for RNA marker II or III).

Recommended RNA target loading amounts

Because the DIG system uses relatively high probe concentrations, low target RNA amounts are needed for detection even for the detection of rare mRNAs.

Please refer to the following table for the recommended loading amounts.

If you are using DNA probes and...	**Then load:**
total RNA	5 µg per lane
mRNA	500 ng per lane
If you are using RNA probes and...	**Then load:**
total RNA	1 µg per lane
mRNA	100 ng per lane

Additional solutions required

Please refer to the following table to find additional solutions required.

Solution	Composition
10 x MOPS, pH 7.0 (with NaOH)	200 mM MOPS (4-morpholine-propanesulfonic acid 50 mM Na-Ac, 10 mM EDTA, pH 7
Loading buffer 1	250 µl formamide (freshly deionised) 83 µl 37% formaldehyde (v/v) 50 µl 10 x MOPS 50 µl glycerol (RNase-free) 10 µl 2.5% bromophenolblue (w/v) 57 µl DEPC/DMPC-treated H_20
Loading buffer 2 (*recommended for loading the samples into the dry wells of the gel)*	250 µl formamide (freshly deionised), 83 µl 37% formaldehyde (v/v), 50 µl 10 × MOPS buffer, 0.01% bromophenolblue (w/v) Adjust to 400 µl with DEPC/DMPC-treated H_20
Gel solution (final volume 100 ml)	0.8 – 2 % agarose 85 ml DEPC/DMPC-treated H_20 10 ml 10 x MOPS 5 ml 37% formaldehyde (v/v)
Running buffer	1 x MOPS
Transfer Buffer	20 x SSC (3 M NaCl, 300 mM Na-citrate, pH 7.0)

Additional material required

- RNase-free electrophoresis chamber and power supply
- Water bath at +65°C
- Micro centrifuge
- Orbital shaker
- Nylon Membrane, positively charged*
- Vacuum blotter (*e.g.*, Pharmacia)
- UV-cross linker (*e.g.*, Stratagene) or hybridization oven
- Whatmann 3 MM paper

Gel preparation

For optimal results we recommend the use of Agarose MP* (Cat. No. 11 388 983 001) from Roche Applied Science.

Step	Action
1	For the preparation of a 100 ml gel, add 10 ml 10 × MOPS buffer and 85 ml H_2O to the agarose.
2	Boil to dissolve the agarose, and allow to cool to +60°C.
3	Perform the following steps under a hood! Add 5 ml 37% formaldehyde (v/v), mix well and immediately pour the gel.

Protocol

In the following protocol the pre-treatment of the marker, electrophoresis and transfer are described.

Step	Action
1	Thaw RNA Molecular Weight Marker I, II or III, Digoxigenin labeled, on ice. ***Note***: Mix well before use.
2	Mix 1 volume RNA Molecular Weight Marker with 4 volumes freshly prepared loading buffer. ***Note:*** Choice of Loading buffer 1 or 2 depends on loading technique (compare to step 5).
3	Incubate at +65 to +70°C for 15 min.
4	Chill the denatured marker immediately on ice for 2 min.
5	Spin down condensated water and load the marker on your gel. ***Note:*** To avoid spillage of samples the gel should not be submerged in the buffer during loading into the dry wells. In this case use Loading buffer 2.
6	• Run the gel in 1× MOPS running buffer for 5 min at a high current (*e.g.*, 100 mA). • Once samples have entered the gel, submerge the gel in 2–3 mm running buffer • Continue electrophoresis at 80 V (50–60 mA) for 2–3 h or at a correspondingly lower voltage overnight.
7	Remove formaldehyde by incubating the gel in 20× SSC for 2× 15 min on an orbital shaker.
8	Transfer the RNA to a nylon membrane, positively charged by o/n capillary transfer or with a vacuum blotter (*e.g.*, 1.5-2 h with 50 mbar).
9	The RNA is fixed onto the membrane by a UV cross-linking or by baking. (Detailed protocol in package insert of DIG Northern Starter Kit, available on our website http://roche-applied-science.com)

2.2 Hybridization and Detection

Hybridization

We recommend to perform the hybridization experiment and stringent washes according to standard protocols available in the package inserts of *e.g.*, DIG Northern Starter Kit* or the DIG Application Manual for Filter hybridization.

Note: Best results are achieved with the hybridization solution DIG Easy Hyb Granules*.

Detection of DIG-labeled RNA

DIG-labeled RNA and the DIG-labeled molecular weight standards are detected by an antibody conjugated to the enzyme alkaline phosphatase* which catalyzes a chemiluminescent or a color reaction. We recommend the combination of the following products:

- DIG Wash and Block Buffer Set* contains washing buffer, maleic acid buffer, blocking solution and detection buffer in 10 x form
- Anti-DIG-AP, Fab fragments*
- Substrates for the Anti-Digoxigenin-AP:
  For fastest results use chemiluminescent substrates:
  CPD-*Star* , ready-to-use*
  CSPD , ready-to-use*

Note: Please follow the instruction sheets of the above mentioned substrates for the detection protocol.

3. Appendix

3.1 References

1 Sambrook, J.; Fritsch, E.F. & Maniatis, T., (1989) in Molecular Cloning, A Laboratory anual, Cold Spring Harbor Laboratory.
2 Mottier, V. et al (2004) *Insect Biochemistry and Molecular Biology* **34,** 51-60.

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage http://www.roche-applied-science.com and our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection:
  http://www.roche-applied-science.com/DIG/

Kits

Product	Pack size	Cat. No.
DIG DNA Labeling and Detection Kit	25 labeling reactions and 50 blots	11 093 657 910
DIG Gel Shift Kit 2nd Generation	1 kit	03 353 591 910
DIG High Prime Labeling and Detection Starter Kit II	12 labeling reactions and 24 blots	11 585 614 910
DIG Luminescent Detection Kit for Nucleic acids	1 kit (50 blots)	11 363 514 910
DIG Northern Starter Kit	1 kit (10 labeling reactions)	12 039 672 910
DIG Nucleic Acid Detection Kit	40 blots (10× 10 cm)	11 175 041 910
DIG PCR Probe Synthesis Kit	25 reactions	11 636 090 910

Single reagents

Product	Pack size	Cat. No.
Protector RNase Inhibitor	2000 U 10 000 U	03 335 399 001 03 335 402 001
Blocking reagent	50 g	11 096 176 001
CDP-*Star*, ready -to-use	2× 50 ml	12 041 677 001
CSPD , ready-to-use	2× 50 ml	11 755 633 001
DIG Easy Hyb (ready-to-use hybridization solution without formamide)	500 ml	11 603 558 001
DIG Easy Hyb Granules	1 set (6× 100 ml)	11 796 895 001
DIG Wash and Block Buffer Set	30 blots (100 cm^2)	11 585 762 001
Lumi-Film Chemiluminescent Detection Film	100 films (18× 24 cm) 100 films (20.3× 25.4 cm)	11 666 916 001 11 666 657 001
NBT/BCIP stock solution	8 ml	11 681 451 001
Nylon Membrane, positively charged (20× 30 cm) (10× 15 cm) (0.3× 3 m roll)	 10 sheets 20 sheets 1 roll	 11 209 272 001 11 209 299 001 11 417 240 001

Changes to previous version

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Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

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