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

DIG RNA Labeling Kit (SP6/T7)

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RNA labeling with digoxigenin-UTP by *in vitro* transcription with SP6 and T7 RNA polymerase.

Cat. No. 11 175 025 910 1 kit
2 x 10 labeling reactions

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG RNA Labeling Kit (SP6/T7), pSPT18 DNA	▪ Solution, 0.25 mg/ml ▪ Cloning/transcription vector; subclones are transcribed into RNA probes by T7 or SP6 RNA polymerase.	1 vial, 40 µl
2	DIG RNA Labeling Kit (SP6/T7), pSPT19 DNA	▪ Solution, 0.25 mg/ml ▪ Cloning/transcription vector; subclones are transcribed into RNA probes by T7 or SP6 RNA polymerase.	1 vial, 40 µl
3	DIG RNA Labeling Kit (SP6/T7), Control DNA 1 pSPT18-Neo	▪ Solution, 0.25 mg/ml , cleaved with Pvu II. ▪ Transcription of control DNA 1 by T7 RNA polymerase according to the standard protocol produces DIG-labeled “antisense” Neo transcripts of 760 bases in length. ▪ Use as control in labeling and hybridization reactions. ▪ Contains DNA fragments of 798 and 3,281 bp due to cleavage at the 2 Pvu II sites on the plasmid.	1 vial, 20 µl
4	DIG RNA Labeling Kit (SP6/T7), Control DNA 2 pSPT19-Neo	▪ Solution, 0.25 mg/ml , cleaved with Pvu II. ▪ Transcription of control DNA 2 by SP6 RNA polymerase according to the standard protocol produces DIG-labeled “antisense” Neo transcripts of 760 bases in length. ▪ Use as control in labeling and hybridization reactions. ▪ Contains DNA fragments of 316 and 3,761 bp due to cleavage at the 2 Pvu II sites on the plasmid (one at position 761 within the neo gene).	1 vial, 20 µl
5	DIG RNA Labeling Kit (SP6/T7), Labeled control RNA	▪ Solution,100 ng/µl ▪ DIG-labeled “antisense” Neo RNA (760 bases), transcribed with T7 RNA polymerase from 4 µl (equivalent to 1 µg) Pvu II-linearized pSPT18-Neo DNA (Vial 3) according to the standard protocol. The transcription assay was not treated with DNase I. After ethanol precipitation, it was dissolved in dimethylpyrocarbonate-treated water. ▪ The solution contains approximately 10 µg of DIG-labeled Neo RNA and 1 µg pSPT18-Neo template DNA. ▪ For semi-quantitative estimation of DIG-labeled RNA and use as a hybridization control. ▪ Contains DNA fragments of 798 and 3,281 bp.	1 vial, 100 µl

6	DIG RNA Labeling Kit (SP6/T7), Unlabeled control RNA	▪ Solution, 200 µg/ml ▪ Unlabeled Neo poly (A) "sense" RNA in dimethylpyrocarbonate-treated water. ▪ The Neo poly (A) RNA is synthesized by *in vitro* transcription and is 1 kb long. ▪ Target RNA to practice RNA/RNA hybridizations; when applied to a membrane, this RNA will hybridize with the Labeled control RNA (Vial 5).	1 vial, 20 µl
7	DIG RNA Labeling Kit (SP6/T7), NTP labeling mixture, 10x conc.	Nucleotide mixture containing 10 mM ATP, 10 mM CTP, 10 mM GTP, 6.5 mM UTP, 3.5 mM DIG-11-UTP, pH 7.5 (+20°C).	1 vial, 40 µl
8	DIG RNA Labeling Kit (SP6/T7), Transcription buffer, 10x conc.	For RNA labeling reaction.	1 vial, 40 µl
9	DIG RNA Labeling Kit (SP6/T7), DNase I, RNase-free	▪ Solution, 10 U/µl ▪ Degrades DNA template after the labeling reaction.	1 vial, 40 µl
10	DIG RNA Labeling Kit (SP6/T7), RNase Inhibitor	▪ Solution, 20 U/µl ▪ Prevents the degradation of RNA during the labeling reaction.	1 vial, 20 µl
11	DIG RNA Labeling Kit (SP6/T7), SP6 RNA Polymerase	▪ Solution, 20 U/µl ▪ Synthesizes RNA from a DNA template.	1 vial, 20 µl
12	DIG RNA Labeling Kit (SP6/T7), T7 RNA Polymerase	▪ Solution, 20 U/µl ▪ Synthesizes RNA from a DNA template.	1 vial, 20 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	pSPT18 DNA	Store at −15 to −25°C.
2	pSPT19 DNA	
3	Control DNA 1 pSPT18-Neo	
4	Control DNA 2 pSPT19-Neo	
5	Labeled control RNA	
6	Unlabeled control RNA	
7	NTP labeling mixture, 10x conc.	
8	Transcription buffer, 10x conc.	
9	DNase I, RNase-free	
10	RNase Inhibitor	
11	SP6 RNA Polymerase	
12	T7 RNA Polymerase	

1.3. Additional Equipment and Reagent required

For DIG RNA labeling

See section, **Working Solution** *for additional information on how to prepare solutions.*

- Water bath
- Water, PCR Grade treated with DMPC
- 0.2 M EDTA, pH 8.0

For determination of labeling efficiency

See section, **Working Solution** *for additional information on how to prepare solutions.*

- Nylon Membranes positively charged*
- UV transilluminator or UV crosslinker
- RNA dilution buffer
- DIG Wash and Block Buffer Set*
- Anti-Digoxigenin-AP, Fab fragments*
- CSPD* or CDP-*Star**
- Lumi-Film*
- DIG Luminescent Detection Kit*

1.4. Application

Use the DIG RNA Labeling Kit (SP6/T7) to label RNA with digoxigenin-UTP by *in vitro* transcription. DIG-labeled RNA is used for hybridization to:

- Northern blots
- Southern blots
- *In situ* hybridization
- Plaque or colony lifts
- RNase protection experiments

As the linkage between DIG and UTP is resistant to alkali, DIG-labeled RNA can be fragmented by alkaline treatment. Slightly reducing the size of the DIG-labeled RNA probe may make it more suitable for certain applications in in situ hybridization.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

- Linearized plasmid DNA
- PCR product

Control Reactions

To check if the DIG-labeled RNA probe has been labeled efficiently, see section, **Protocols, Determination of labeling efficiency**.

Optional: In vitro transcription of linearized control plasmid (Vials 3 or 4) is possible using T7 or SP6 RNA Polymerase according to the standard protocol.

General Considerations

Precautions

- Work under clean and RNase-free conditions.
- Autoclave DIG System solutions.
- Filter-sterilize solutions containing SDS.
- Do not add Tween 20* to solutions until after they have been sterilized.
- Rigorously clean and rinse laboratory trays before each use.
- When performing northern blots, use sterilized glass trays and solutions for all washing and detection steps.
- Wear powder-free gloves when handling membranes.
- Handle membrane only on the edges and with clean forceps.

Template DNA requirements

Feature	Detail
Purity	▪ For plasmid DNA, use the High Pure Plasmid Isolation Kit* for purification. ▪ For purification of the linearized template, use the High Pure PCR Product Purification Kit*.
Size	▪ To obtain optimal results, template DNA should be linearized and should have a size of ≥100 bp. ▪ Template DNA >10 kb should be restriction digested using a 4 bp cutter prior to labeling.
Amount	The RNA labeling protocol can produce up to 10 µg of labeled RNA from 1 µg of plasmid DNA template. *Larger amounts of DNA can be labeled by scaling up of all components and volumes.*

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation/ Composition	Storage and Stability	For use in...
Water, PCR Grade* or DMPC-treated water	Double-distilled water treated with 0.1% methylpyrocarbonate.	Store at +15 to +25°C.	Adjusting the reaction volume and/or resuspending RNA.
EDTA	0.2 M ethylenediaminetetraacetic acid, pH 8.0		Stopping the reaction.
RNA dilution buffer	Mix RNase-free, DMPC-treated double-distilled water:20x SSC:formaldehyde in the ratio 5:3:2.	⚠ ***Always prepare fresh.***	Dilution series

2.2. Protocols

Sample preparation

1. Linearize the DNA template by cutting at a restriction enzyme site downstream from the cloned insert.
   – To avoid transcription of undesirable sequences, use a restriction enzyme that creates 5′ overhangs.

2. After restriction digest, purify the DNA with the High Pure PCR Product Purification Kit* or via phenol/chloroform extraction and subsequent ethanol precipitation.

Standard RNA labeling reaction

1. Add 1 µg purified template DNA or 4 µl control DNA (Vials 3 or 4) to a RNase-free reaction vial.
   – Add enough Water, PCR Grade* or DMPC-treated water to the vial up to a total sample volume of 13 µl.

2. Place the reaction vial on ice and add the following reagents:

Component	Volume [µl]
10x NTP labeling mixture (Vial 7)	2
10x Transcription buffer (Vial 8)	2
RNase Inhibitor (Vial 10)	1
RNA Polymerase SP6 or RNA Polymerase T7 (Vials 11 or 12)	2

   – Mix gently and centrifuge briefly.
   – Incubate for 2 hours at +37°C.
   Longer incubations do not increase the yield of labeled RNA.

3. As an optional step, add 2 µl DNase I, RNase-free to remove template DNA.
   Recommended for RNase-protection experiments.
   – Incubate for 15 minutes at +37°C.

4. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0.

The RNA transcripts can be analyzed by agarose gel electrophoresis, for example, using formaldehyde gels or native gels, and ethidium bromide staining.

DNase treatment

If you are using the DIG-labeled RNA for hybridization to northern or Southern blots or plaque or colony lifts, you do not need to treat the sample with DNase (optional Step 3 above), since the amount of DIG-labeled RNA transcript is far in excess of the template DNA.

Handling and storage of labeled probe

1. Do not perform a phenol/chloroform extraction with your DIG-labeled probe because it will partition into the organic phase.

2. Store labeled probes a −15 to −25°C for a minimum of 1 year.

⚠ ***Avoid repeated freezing and thawing of the labeled probe.***

Scaled up reaction

Reaction may be scaled up to increase the yield of labeled RNA.

1. Keep the amount of template DNA constant while increasing the amount of the other components in the labeling reaction to generate larger amounts of labeled probe.

2. In a 5x scaled-up reaction with 1 µg linear Control DNA (Vial 3) as template, you can synthesize more than 40 µg of DIG-labeled RNA in a 2 hour incubation at +37°C.

Hybridization

1. For northern analysis, load 1 µg of total RNA or 100 ng mRNA onto the gel.
   – DIG-labeled RNA can be used in standard hybridization protocols.
   – Use of DIG Easy Hyb buffer* will decrease the hybridization time to <6 hours and the amount of hybridization background.

2. Use 20 to 100 ng of the DIG-labeled RNA probe per ml hybridization mix.
   – For best results, use Nylon Membranes, positively charged* which are function tested for optimal results with the DIG System.

3. Store hybridization solutions containing labeled RNA at −15 to −25°C.
   – The solutions can be reused provided that all solutions are RNase-free.
   – If using DIG Easy Hyb buffer, denature the probe for 10 minutes at +65°C before using it again.

⚠ ***Do not boil.***

Immunological detection

1. Analyze the hybridized membrane immediately using the immunological detection procedure, or store the membrane sealed in a plastic bag for later analysis.
   – The detection procedure involves blocking the membrane and then using the Anti-DIG-AP conjugate* to detect DIG-labeled RNA on the membrane.

2. Use a chemiluminescent substrate, such as CSPD* or CDP-*Star*® to visualize the antibody-probe complexes on the membrane.

3. Expose the membrane to an imaging device or X-ray film or Lumi-Film* for 10 to 30 minutes.

4. The membranes can be stripped and reused for hybridization, provided that the membrane does not dry out at any time and is handled under RNase-free conditions throughout the procedure.

i A detailed detection procedure is included in the DIG Luminescent Detection Kit for easy and sensitive detection of DIG-labeled RNA probes.*

Determination of labeling efficiency

It is important to determine the yield of DIG-labeled RNA for optimal and reproducible hybridization results. Using too much probe in the hybridization mix causes background, while using too little concentration leads to weak signals. The preferred method for quantification of labeled probes is direct detection via spot test.

Direct detection

1. Apply a series of dilutions of DIG-labeled RNA to a Nylon Membrane, positively charged*.

2. Spot several known dilutions of the Labeled control RNA (Vial 5) onto the membrane.
   – These dilutions serve as standards.

3. Perform an immunological detection reaction on the membrane with Anti-Digoxigenin-AP conjugate* and the premixed stock solution of CSPD ready-to-use* or CDP-*Star*®.

4. Expose the membrane to an imaging device or X-ray film or Lumi-Film* to visualize the spots.

Preparation of probe stock solution for the spot test

The efficiency of the labeling reaction depends on the starting amount of template and the incubation time.

1. To perform the spot test, initially assume that the yield of the labeling reaction was 10 µg of labeled RNA/1 µg of template.

2. Dilute your labeled probe accordingly to a starting concentration of 10 ng/µl.

3. Dilute the DIG-Labeled control RNA (Vial 5) to 10 ng/µl.

4. Use these starting solutions to prepare the following dilution series.

Dilution series

Prepare a dilution series of your labeled probe and your control RNA as described in the following table:

⚠ ***Highly diluted solutions of RNA in water are not stable. Spot the dilutions on the test membrane immediately after preparing them.***

Tube	RNA [µl]	From tube No.	RNA dilution buffer [µl]	Dilution	Final concentration
1	–	Dilution of probe and Vial 5	–	–	10 ng/µl
2	2	1	18	1:10	1 ng/µl
3	2	2	198	1:100	10 pg/µl
4	15	3	35	1:3.3	3 pg/µl
5	5	3	45	1:10	1 pg/µl
6	5	4	45	1:10	0.3 pg/µl
7	5	5	45	1:10	0.1 pg/µl
8	5	6	45	1:10	0.03 pg/µl
9	5	7	45	1:10	0.01 pg/µl
10	0	–	50	–	0

Spot test

1. Apply 1 µl spots of tubes 3 to 10 from your labeled probe and the labeled control RNA to a strip of Nylon Membrane.

2. Fix the nucleic acid to the membrane by crosslinking with UV light or baking for 30 minutes at +120°C.

3. Follow the chemiluminescent detection procedure described in the Instructions for Use of the DIG Luminescent Detection Kit* or the substrates CSPD* or CDP-*Star**.
   – Use volumes appropriate to the size of your membrane strip.

ⓘ *The colorimetric detection procedure using NBT/BCIP* is described in the Instructions for Use of the DIG Nucleic Acid Detection Kit*.*

Analyzing the result

Compare the intensity of the DIG-labeled probe spots to that of the control spots and calculate the amount of DIG-labeled RNA.

2.3. Parameters

Sensitivity

DIG-labeled RNA probes can detect single-copy genes in as little as 1 µg of mammalian DNA under the following assay conditions:

- The hybridization mix contains 20 to 100 ng labeled probe/ml.
- The bound probe is detected with Anti-DIG-AP, Fab fragments and visualized with the chemiluminescent substrate CDP-*Star*.

3. Results

Labeling efficiency

The amount of synthesized labeled RNA depends on the amount, size (site of linearization), and purity of the template DNA.

- Under standard conditions, approximately 10 µg of full-length DIG-labeled RNA will be transcribed from 1 µg template DNA.
- When 1 µg of linearized template DNA (Vials 3 or 4) is labeled in the standard reaction, approximately 37% of the nucleotides are incorporated into about 10 µg of DIG-labeled RNA (transcript length, 760 bases).

4. Troubleshooting

Observation	Possible cause	Recommendation
Low sensitivity observed.	Inefficient probe labeling.	Check labeling efficiency; the labeling reaction can be scaled up.
		Purify template DNA using the High Pure PCR Product Purification Kit* or phenolization.
		Make sure that the template was linearized before labeling.
		Do not store template in buffers containing >0.1 mM EDTA.
		Check the amount and quality of target RNA or DNA.
	Low probe concentration in the hybridization.	Increase probe concentration to 100 ng/ml.
		Prolong hybridization time to overnight.
	Target degradation, weak signals in northerns blots.	Work under RNase-free conditions.
		Use the DIG Wash and Block Buffer Set*.
High background present.	Concentration of labeled probe is too high.	Quantify your labeled probe via a spot test, see section, **Dilution series**. ⚠ ***Do not use >100 ng/ml hybridization mix.*** ▪ Decrease probe concentration. ▪ Make sure that the membrane was soaked in sufficient prehybridization solution.
	Wrong type of nylon membrane.	Some types of nylon membrane may cause high background. Use Nylon Membranes, positively charged* especially tested for the DIG System. ⚠ ***Do not allow the membrane to dry at any time.***
	Ineffective stringency washes.	Check temperature of stringency washes; prewarm wash solution to correct temperature.
		Eventually decrease stringency wash from 0.5x SSC to 0.1x SSC.
Smear in lanes	Target concentration too high.	For a northern blot, do not load >1 µg of total or 100 ng of mRNA per lane. ⓘ *The DIG System is very sensitive compared to radioactivity and higher RNA concentrations result in detection of degradation products.*

5. Additional Information on this Product

5.1. Test Principle

Labeling principle

The DIG RNA Labeling Kit produces DIG-labeled, single-stranded RNA probes of known length. Either SP6 or T7 RNA polymerase transcribes these probes *in vitro* from template DNA in the presence of digoxigenin-UTP.

① During RNA labeling by in vitro transcription, DNA to be transcribed is cloned into the polylinker site of appropriate transcription vectors, such as pSPT18 or 19, which contain promoters for SP6 and T7 RNA polymerases.
– Adjacent template DNA is linearized at a suitable site.
– RNA polymerases are used to produce “run off” transcripts.
– DIG-UTP is incorporated into the transcript; every 20 to 25th nucleotide of the newly synthesized RNA is a DIG-UTP.
– Since the nucleotide concentration does not become limiting in the standard transcription reaction, this reaction can generate large amounts of labeled RNA.

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② When used in standard procedures, DIG-labeled probes will hybridize to nucleic acids on a blot or *in situ*.

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③ DIG-labeled RNA probes can be detected with Anti-Digoxigenin-AP (Fab fragments conjugated to alkaline phosphatase).
– Bound antibody conjugate is then visualized with the highly sensitive chemiluminescent substrates CSPD or CDP-*Star* (filter hybridization) or NBT/BCIP (*in situ*).

Cloning into pSPT18/19

The pSPT18/19 plasmids are pUC derivatives, containing the pBR322 origin and the amp gene, but no *lac* operon (Fig 1). Therefore, the presence of these plasmids cannot be detected by blue/white selection methods.

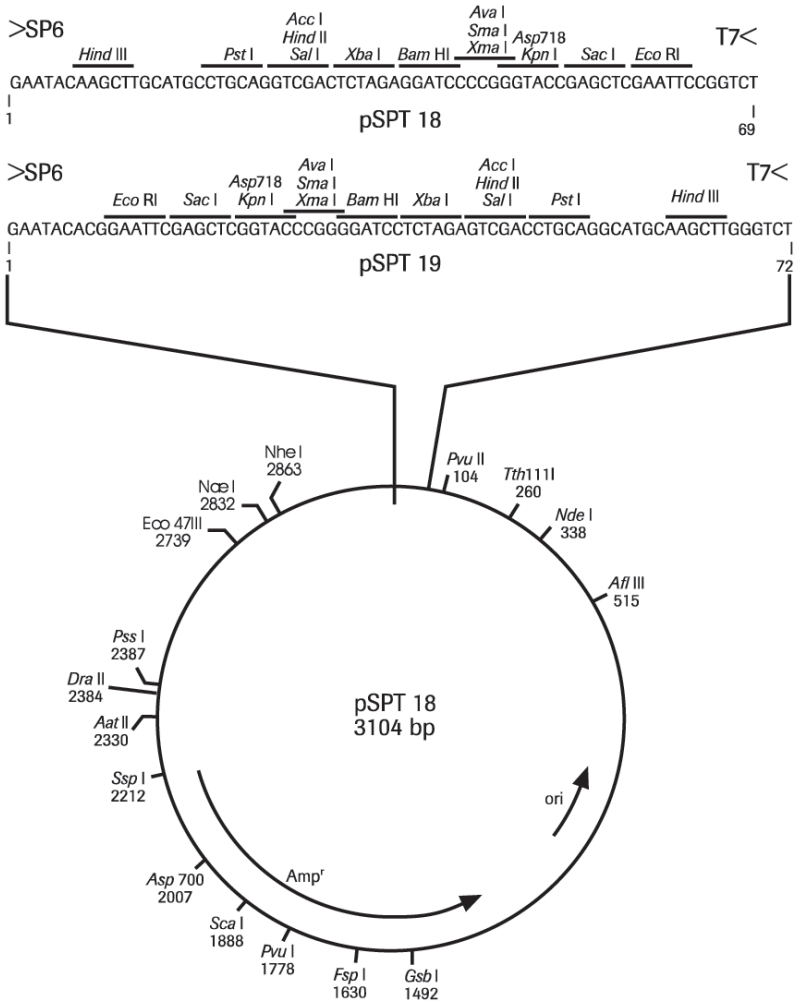

Fig. 1: Polylinker sites of the transcription Vectors pSPT18 and pSPT19.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Nylon Membranes, positively charged	10 sheets, 20 x 30 cm	11 209 272 001
	20 sheets, 10 x 15 cm	11 209 299 001
	1 roll, 0.3 x 3 m	11 417 240 001
DIG Wash and Block Buffer Set	1 set, 30 blots (100 cm^2)	11 585 762 001
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
Lumi-Film Chemiluminescent Detection Film	100 films, 7.1 x 9.4 inches, 18 x 24 cm, *Not available in US*	11 666 916 001
DIG Luminescent Detection Kit	1 kit, 50 blots with a size of 10 x 10 cm^2	11 363 514 910
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
High Pure Plasmid Isolation Kit	1 kit, 50 purifications	11 754 777 001
	1 kit, 250 purifications	11 754 785 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
DIG Easy Hyb	500 ml	11 603 558 001
NBT/BCIP Stock Solution	8 ml	11 681 451 001
DIG Nucleic Acid Detection Kit	1 kit, Detection of 40 blots of 10 cm x 10 cm	11 175 041 910

6.4. Trademarks

DIG EASY HYB is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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