GE Healthcare

Amersham
Nick Translation Kit

Product Booklet

Codes: N5000 For radioactive probe preparation
N5500 For radioactive and non-radioactive probe preparation

Page finder

1. Legal

http//www.gehealthcare.com/lifesciences

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only.* Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Caution: For use with radioactive material.
This product is to be used with radioactive material. Please follow the manufacturer's instructions relating to the handling, use, storage and disposal of such material.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at -15°C to -30°C in a non frost-free freezer.

2.3. Stability

Stable for 3 months, stored as recommended.

3. Components of the system

Nick translation systems	N 5000	N 5500
Nucleotide/buffer solution; 100 mM each of dATP, dGTP and dTTP in Tris-HCl pH 7.8, 2 mercaptoethanol, and $MgCl_2$	400 µl	–
Nucleotide solutions; in Tris-HCl pH 7.8, 2-mercaptoethanol and $MgCl_2$		
300 µM dATP	–	150 µl
300 µM dCTP	–	150 µl
300 µM dGTP	–	150 µl
300 µM dTTP	–	150 µl
Enzyme solution; 0.5 units/µl DNA polymerase I and 10 pg/µl DNase I in Tris-HCl pH 7.5, $MgCl_2$, glycerol and bovine serum albumin	200 µl	200 µl
Standard DNA solution; 200 ng/µl *Hin*d III digested lambda DNA in 10 mM Tris-HCl pH 8.0, 1 mM EDTA	25 µl	25 µl
Water	2 x 1 ml	2 x 1 ml

4. Description

The nick translation kits from GE Healthcare utilize the nick translation reaction (1,2), catalyzed by *E.coli* DNA polymerase I, to introduce radioactively and non-radioactively-labelled nucleotides into DNA. The enzyme has three distinct activities, a template-dependent DNA polymerase activity acting in the 5′-3′ direction, a 5′-3′ exonuclease activity and a 3′-5′ exonuclease activity. The nick translation reaction uses the ability of DNA polymerase I to couple the first two of these activities so as to add nucleotides sequentially to the 3′-end of a nick within a DNA duplex, while removing nucleotides from the adjacent 5′-terminus. Nicks are introduced in a random manner by the action of pancreatic deoxyribonuclease I (DNase I), so that the nett effect is to produce a uniformly-labelled population of molecules. In nick translation, the existing nucleotide sequence in the DNA is renewed without nett synthesis occurring. At the standard incubation temperature of 15°C, the maximum extent of the reaction is theoretically limited to one complete replacement of the existing nucleotide sequence. Any double-stranded DNA molecule can be used as substrate.

N5000 contains non-radioactive components for use with radioactively labelled dCTP.

N5500 contains non-radioactive components for use with any radioactively labelled dNTP.

Both kits are recommended for use with the appropriate Redivue™ nucleotides.

N5500 can also be used with non-radioactively labelled nucleotides, for example Cy™3- or Cy5-dCTP.

Radioactive probe labelling

If the replacement nucleotides inserted during the process are radioactive, the DNA will become labelled to a specific activity determined by the extent to which the existing nucleotides are replaced and by the specific activity of the newly inserted nucleotides. It is rare for more than approximately 75% of the labelled nucleotide to be incorporated and an incorporation of 40–60% is a more typical figure. If insufficient nucleotide to allow complete replacement of the existing sequence is used, the proportion of the existing nucleotide replaced is reduced and the resulting labelled DNA is of lower specific activity. However, by using high specific activity nucleotides (for example ^{32}P-dNTPs at 3000 Ci/mmol), it is possible to obtain high specific activity probes (>10^8 dpm/µg) even with partial replacement of nucleotides, for example, when using the nick translation reactions to label microgram quantities of DNA. In addition, similar levels of high specific activity nucleotides can be used with lower amounts of DNA (50–100 ng), giving higher levels of replacement and of probe specific activity (10^9 dpm/µg), although the amount of probe is correspondingly reduced. In this booklet, protocols are given for radioactively labelling 1 µg and 50 ng DNA, but it is also possible to label intermediate amounts of DNA with relatively little alteration to the reactions given. Nucleotides labelled at the alpha-phosphate position with ^{32}P, ^{33}P or ^{35}S can be incorporated, as can ^{3}H-labelled nucleotides. The radiolabelled nick translation products of this system are generally greater than 500 bp in length.

Non radioactive probe labelling

Non radioactively labelled nucleotides labelled with the new CyDye™ fluors, for example Cy3- and Cy5-dCTP, or traditional fluors, for example fluorescein-, rhodamine- and coumarin-dUTP, can also be incorporated (3). The standard reaction produces probes greater than 500 bp in length. However, if nick translated DNA is to be used

for *in situ* hybridization experiments probes of 500 bp or less are optimal, to allow the DNA access to the target, such as nuclear DNA. Consequently, a modification to the standard protocol is required for the production of probes for *in situ* hybridization and this is presented in the section on labelling of probes with non-radioactive nucleotides. Protocols are for labelling 1 µg of DNA , but it is also possible to perform 0.5 µg labelling reactions, and they can be adapted for labelling with other non-radioactively modified nucleotides.

5. Critical parameters

- Read the protocol thoroughly before starting.
- Note that additional DNase I will be required for the production of non radioactive *in situ* hybridization probes. As only very small amounts of DNase I are required, careful control of the addition of this extra amount of enzyme is necessary.
- Prepare any additional solutions in advance (see page 10).
- Careful control of the temperature of the nick translation reaction is necessary to avoid the generation of 'snap back' regions in the labelled DNA (4).

6. Additional solutions and reagents required

Solutions

SDS (sodium dodecyl sulphate)
Prepare a 10% (w/v) stock solution

2xSSC
30 mM Na_3 citrate, pH7
300 mM NaCl

TE buffer
10 mM Tris-HCl, pH 8
1 mM EDTA

EDTA stop solution
0.2 M EDTA, pH 8

DNase I dilution buffer
150 mM NaCl

Reagents
Ethanol
DNase I

7. Nick translation system protocols

7.1. Storage and stability

Upon receipt of these kits, components should be stored at -15°C to -30°C. Do not store in a frost-free freezer. The kit components are stable for at least 3 months when stored under the recommended conditions.

All labelled probes should be stored at -15°C to -30°C. The length of time that is appropriate for the storage of radiolabelled probes will largely be determined by the isotope used in the labelling reaction. Non-radioactively labelled probes should be stable for at least 3 months provided fluor labelled probes are stored in the dark.

7.2. Large scale preparation of radioactively labelled probe

The protocol given is for the use of 33 pmoles of [α-^{32}P]dNTP, specific activity 3000 Ci/mmol. For N5500 this can be any [α-^{32}P] labelled nucleotide, for example Redivue codes AA 0004 - AA 0007. For N5000 [α-^{32}P]dCTP (Redivue code AA 0005) is used.

This protocol typically generates probes of specific activity 1-2 x 10^8 dpm/µg*.

*At the activity reference date of the labelled nucleotide.

Protocol	Notes
1. Dilute the DNA to be labelled to a concentration of 20–200 ng/µl in either distilled water or TE buffer.	**1.** Concentrate dilute DNA solutions by ethanol precipitation and redissolve in an appropriate volume of water or TE buffer.

Protocol	Notes
2. Place the required tubes from the nick translation kit, with the exception of the enzyme solution, at room temperature to thaw. Once thawed place on ice.	**2.** Leave the enzyme solution at -15°C to -30°C until required, and return to the freezer immediately after use.
3. Place the DNA sample (1 µg) into a clean microcentrifuge tube and add the nucleotide/ buffer solution, radiolabelled dNTP, water and enzyme solution;	**3.** When using the nick translation system N5500 prepare, in a separate clean microcentrifuge tube, the appropriate nucleotide/buffer mix as indicated below;

Component	N 5000	N 5500	Labelled dNTP to be used	Volume (µl) unlabelled dNTP required in mix			
Template DNA	1 µg	1 µg		dATP	dCTP	dGTP	dTTP
Nucleotide/buffer	20 µl	20 µl (see note 3)	dATP	--	7	7	7
[α-^{32}P]dCTP (AA0005)	10 µl	---	dCTP	7	--	7	7
[α-^{32}P]dNTP (AA0004-7)	---	10 µl	dGTP	7	7	--	7
Water to ensure a final reaction volume of 100 µl			dTTP	7	7	7	--
Enzyme solution	10 µl	10 µl	Use 20 µl of this mix in the standard labelling reaction				

4. Mix gently by pipetting up and down, and cap the tube. Spin for a few seconds in a microcentrifuge to bring the contents to the bottom of the tube.	**4.** Avoid vigorous mixing of the reaction mixture as this can cause severe loss of enzyme activity.
5. Incubate at 15°C for 2 hours.	

Protocol	Notes
6. Stop the reaction by the addition of 10 µl of 0.2 M EDTA pH 8.0. The labelled probe is now ready for use in hybridization following denaturation at 95–100°C for 5 minutes.	**6.** The reaction may be monitored if required as detailed on page 18. Labelled probe can be stored at -15°C to -30°C in a non frost-free freezer.

Additional notes and troubleshooting guidance

- Small scale preparation of radioactively labelled probe.

  This protocol typically generates probes of specific activity 1–1.5 x 10^9 dpm/µg (at the activity reference date of the labelled nucleotide). DNA fragments purified in low melting point agarose may also be labelled using this protocol.

  Use 50 ng template. Prepare the same nucleotide mix as for the standard reaction but on a half scale. Add only 10 µl of the mix to the reaction along with half amounts of enzyme and radioactive nucleotide. Add water to a final volume of 50 µl. Incubate for 60 minutes and add 5 µl 0.2 M EDTA to stop the reaction.

- These protocols may be easily adapted to allow the use of various labelled dNTPs. Table 1 lists a number of labelled dNTPs available from GE Healthcare and shows the highest practicable amounts of label that can be used in a large scale reaction to maximize probe specific activity. In most cases lower amounts of label will be adequate and high specific activity label will be incorporated more rapidly than an equivalent amount of low specific activity label when measured in µCi. When designing a nick translation protocol with the nucleotide of your choice consider the application and the required probe specific activity.

- Care should be taken to ensure that all impurities which might interfere in the labelling reaction have been removed from the DNA.
- This protocol may also be used to label up to 2 µg of DNA. Under these conditions the volume of [α-^{32}P]dNTP solution should be increased to 20 µl (66 pmoles).
- If desired the labelling efficiency of a DNA sample can be compared with that of the standard DNA supplied with the kit. In this case, 5 µl of standard DNA should be used.
- For most labelling reactions 10 µl of enzyme solution will ensure satisfactory labelling within 1–2 hours. However the rate at which radioactivity is incorporated into the DNA will increase with increased enzyme concentration. It may be desirable to add more than 10 µl when high dNTP concentrations are being used to obtain high specific activity probe DNA. The time course of the nick translation reaction with [^{35}S]dCTPαS is somewhat slower than with the natural nucleotides.
- Prolonged storage of ^{32}P-labelled probes can lead to substantial probe degradation. High specific activity probes should be stored for no longer than 3 days.
- Although probe purification is not usually necessary for most membrane applications, the removal of unincorporated nucleotide is sometimes useful to reduce background in filter hybridizations for probes >l0^{9} dpm/µg or when the reactions yield an incorporation of <50%. This procedure is described on page 23. Extensive experimentation with Rapid-hyb™ buffer (RPN1635/6) has shown that probe purification, even under the conditions given above, is not required with the isotopes ^{32}P and ^{33}P. Purification with ^{35}S-labelled probes is however required to reduce filter background.

Table 1. Use of alternative labelled dNTPs for nick translation.

				When used in large scale reaction	
Nuclide	**Compound**	**Specific Activity Ci/mmol**	**Product code**	**µCi required/ µg DNA**	**potential DNA specific activity dpm/µg**
^{32}P	[α-^{32}P]dNTP	400	AA0064-7	100	1×10^8
		800	AA0084-7	200	2×10^8
		3000	AA0004-7	750	7.5×10^8
		6000	AA0074-5	1500	1.5×10^9
^{35}S	[^{35}S]dNTPaS	400	SJ264	100	1×10^8
		600	SJ304-5	150	1.5×10^8
		1000	SJ1304-5	250	2.5×10^8
^{3}H	[1',2',5-^{3}H] dTTP	50-85	TRK625	12.5–17.5	$1.2\text{-}1.8 \times 10^7$
^{33}P	[α-^{33}P]dNTP	1000-3000	AH9904-5	250	$>2 \times 10^8$

7.3. Preparation of non-radioactively labelled probes (using N5500)

This protocol is suitable for the incorporation of Cy3- or Cy5-dCTP, fluorescein-, rhodamine- or coumarin-dUTP into DNA probes. It is also appropriate for other modified nucleotides used in non-radioactive detection. To generate probes specifically for *in situ* hybridization applications, additional DNase I is required with respect to the standard protocol (GE Healthcare products E2210Y or E2210Z). **As only very small amounts of DNase I are required, careful control of the addition of this extra amount of enzyme is necessary.**

Protocol	Notes
1. Dilute the DNA to be labelled to a concentration of 20–200 ng/µl in either distilled water or TE buffer.	**1.** Concentrate dilute DNA solutions by ethanol precipitation and redissolve in an appropriate volume of water or TE buffer.
2. Place the required tubes from the nick translation kit, with the exception of the enzyme solution, at room temperature to thaw. Once thawed place on ice.	**2.** Fluorescent-labelled nucleotides should be protected from light while thawing. Leave the enzyme solution at -15°C to -30°C until required, and return to the freezer immediately after use.
3. Place the DNA sample into a clean microcentrifuge tube and add the nucleotides/ reaction buffer, water and enzyme solution as below:	**3.** When using the nick translation system N5500 prepare, in a separate clean microcentrifuge tube, the appropriate nucleotide/buffer mix as indicated below:

Component	N 5500	Fluor labelled dCTP		Fluor labelled dUTP	
Template DNA	1 µg	dATP	7 µl	dATP	7 µl
Nucleotide/buffer	20 µl (see note 3)	dGTP	7 µl	dGTP	7 µl
Fluor labelled nucleotide (1mM)	1 µl	dTTP	7 µl	dCTP	7 µl
Water to ensure a final volume of 50 µl		dCTP	2 µl	dTTP	2 µl
Enzyme mix	10 µl	Use 20 µl of this mix in the standard labelling reaction			

4. For ISH probes only, add 1 µl of freshly diluted DNase I (0.002333 units of enzyme); representing a 1/30000 dilution of 70 U/µl stock	**4.** Accurately prepare a dilution series of stock DNase I, in 150 mM NaCl, to achieve the required level for addition to the reaction.

Protocol	Notes
4. *Continued.* (E2210Y, GE Healthcare).	
5. Mix gently by pipetting up and down, and cap the tube. Spin for a few seconds in a microcentrifuge to bring the contents to the bottom of the tube.	**5.** Avoid vigorous mixing of the reaction mixture as this can cause severe loss of enzyme activity.
6. Incubate at 15°C for 4 hours.	
7. Stop the reaction by the addition of 5 µl of 0.2 M EDTA pH 8.0. The labelled probe is now ready for use in hybridization following denaturation at 95–100°C for 5 minutes.	**7.** The reaction may be monitored if required as detailed on page 19. Labelled probe can be stored, protected from light, at -15°C to -30°C in a non frost-free freezer.

Additional notes and troubleshooting guidance

- It is possible to use some non-isotopic labelled probes without purification but, if required, probes can be purified by standard techniques such as gel filtration and ethanol precipitation.
- The longer incubation time allows for the slightly slower incorporation of modified nucleotides.
- 0.5 µg labelling reactions can be performed using half the volume of reagents used in the standard labelling reaction.
- Other non-isotopic labelled nucleotides can be used in a direct substitution for the fluor labelled dCTP or dUTP as appropriate.

8. Additional information

8.1. Monitoring incorporation

8.1.1. Radiolabelled nucleotides

Monitoring of the progress of the labelling reaction and measurement of probe specific activity can be achieved by determining the proportion of the radiolabelled nucleotide incorporated by use of an appropriate incorporation assay.

A basic assay is described:

1. Remove 2 µl from the reaction mix to a fresh tube. Add 18 µl water and mix thoroughly by pipetting up and down.
2. Spot 2 µl aliquots on to four 1 cm^2 pieces of Whatman™ DE81 paper (marked with a pencil) placed on a non-absorbent surface.
3. Place two DE81 squares into 2xSSC and wash, with gentle agitation, for 5 minutes. Replace 2xSSC and wash for a further 5 minutes.
4. Rinse washed squares briefly in water and then wash in ethanol for 1 minute.
5. Place washed squares on an absorbent material and allow to air dry.
6. Use a scintillation counter to determine the level of radioactivity on each of the four DE81 squares. Cerenkov counting can be used for ^{32}P labelled samples (counting the dry filters in the ^{3}H channel without scintillant) whilst the squares should be placed in scintillation fluid for all other isotopes.
7. The percentage incorporation can be calculated from the values obtained with results typically exceeding 50% depending on the isotope and reaction conditions used. The specific activity of the probe can also be determined and this figure can be used to gauge the amount of probe to add to a subsequent hybridization procedure.

An alternative assay format would be to perform thin layer chromatography of the labelled probe mix on PEI cellulose TLC plates in 1.25 M KH_2P0_4, pH 3.4, in which the labelled probe is separated from the unincorporated nucleotides. The plates can be scanned directly in an appropriate machine or visualized through the use of a suitable phosphorimager devices. The software will enable the required calculations to be performed.

8.1.2. Non-radioactively labelled nucleotides
Fluorescent labelled nucleotides

Probes labelled with fluorescent nucleotides such as fluorescein-, rhodamine- and coumarin-dUTP can be assessed using the following assay procedure. This assay makes direct use of the physical properties of the fluorescent group. By reference to a set of standards, it is possible to obtain a semi-quantitative estimate of reaction efficiency. Cy3- and Cy5-labelled dCTP labelled probes have not been fully evaluated in this procedure; Cy5 nucleotides exhibit non-specific binding to membranes making assessment of probe labelling difficult.

Protocol	Notes
1. Prepare 1/5, 1/50, 1/100, 1/250, 1/1000 and 1/2500 dilutions of the nucleotide mix in TE buffer.	
2. Dot out 5 µl of labelled probe and 5 µl of the 1/5 dilution of nucleotide mix (negative control) on to a strip of Hybond™ N+, placed on a non-absorbent backing. Allow the liquid to absorb (1 minute), but do not allow	**2.1.** If the spots dry out it may not be possible to wash off the unincorporated nucleotide making interpretation of the labelling result difficult. Prepare the reference strip (step 3) while the wash step is proceeding.

Protocol	Notes
2. *Continued.* to dry. Wash the strip with gentle shaking in excess pre-warmed 2xSSC at 60°C for 15–20 minutes.	**2.2.** Whatman DE81 paper can be used as an alternative to Hybond N+. In this case a 1 cm edge should be left to the filter to avoid damage to the dots during washing and 2xSSC, 0.1% (w/v) SDS should be used to wash the strip. Following the wash step, rinse the strip briefly in water and then wash for 1 minute in ethanol before handling (DE81 is fragile in aqueous solutions). The reference strip can be similarly wetted successively in water and ethanol to reduce distortion to the filter.
3. Prepare a reference strip by dotting 5 µl of each nucleotide mix dilution, except the 1/5, onto a separate strip of Hybond N+ and leave until required.	**3.** The reference strip can be re-used and can be stored, wrapped in SaranWrap™, for several weeks at -15°C to -30°C in the dark.
4. At the end of the wash procedure, place both the reference and washed strips on to a piece of Whatman 3 MM paper lightly moistened	

Protocol	Notes
4. *Continued.* with TE buffer and take to the dark room.	
5. Visualize the strips (DNA side down) on a UV transilluminator; optimum contrast is obtained using a short wavelength (254 nm) transilluminator. The labelled probe should be visible as a fluorescent spot. The strips can be photographed using an appropriate Kodak™ Wratten™ filter and Polaroid™ 667 black and white film, or by scanning on a Molecular Dynamics fluorescence scanning instrument following the guidelines supplied with the instrument.	
6. If the probe clearly shows a brighter spot than the negative control then some probe labelling has occurred; the negative control should retain little or no fluorescence indicating that the fluorescence of the probe is due only to the incorporated fluor.	**6.** If the negative control retains significant levels of signal it may make the interpretation difficult. It is possible to wash the filters for a further 15 minutes if this occurs but it may indicate that the filters were left for too long after spotting out the samples and

Protocol	Notes
	6. *Continued.* that the material has dried on to the filter. In this situation the test would have to be repeated if there was still some doubt about the probe labelling reaction.

Other non radioactive nucleotides

Nucleotides labelled with biotin or a hapten are generally used as indirect labels requiring a secondary detection system which most frequently involves an enzyme labelled moiety that is able to bind to the hapten. The signal is then generated by enzymatic action on a suitable colorimetric or chemiluminescent substrate (5). Following nick translation, labelling efficiency can be determined by preparing dot blots of the probe at suitable dilutions, followed by detection. The most appropriate dilutions are determined by the sensitivity of the detection method employed. If this is unknown, the range 1 pg–100 pg nick translated DNA per dot is a useful starting point. By including a control labelled probe and comparing the intensity of the signals generated the efficiency of labelling may be determined in a semi-quantitative manner.

Size analysis of probes for *in situ* hybridization

1. Prepare a 2%(w/v) agarose gel in TAE ideally using SDF agarose (for separation of small DNA fragments (GE Healthcare, RPN1881).
2. Purify nick translation probes to remove unincorporated nucleotides by gel filtration or ethanol precipitation.
3. Electrophorese purified sample alongside appropriate DNA size markers.
4. Analyze gel directly on a fluorescence scanning instrument or, after staining with ethidium bromide, on a UV transilluminator.

8.2. Removal of unincorporated nucleotides

Removal of unincorporated nucleotides is sometimes desirable to reduce background produced by the probe during hybridization, particularly if the radioactive probe is to be kept for several days before use or the incorporation is less than 50%. If ^{32}P-labelled probes are to be used in combination with GE Healthcare's Rapid-hyb buffer (RPN1635/6), purification is not required unless the probe

is to be used more than 24 hours after preparation. Probes can be purified by gel filtration chromatography or selective precipitation and these methods can be applied to radioactive and non radioactive probe labelling reactions.

8.2.1. Spin columns (6,7)
Probe reactions are passed through columns packed with a gel filtration matrix, which retains the free nucleotides within a column matrix. A number of pre-packed columns are commercially available.

8.2.2. Selective precipitation of labelled DNA
This leads to precipitation of DNA greater than about 20 nucleotides in length with unincorporated nucleotides remaining in solution. Recovery of the labelled DNA by this method varies according to the DNA concentration and size, and may be as low as 50%. Add one volume of 4 M ammonium acetate, pH 4.5 to the nick translation reaction, and mix gently by pipetting up and down. Add four volumes of ethanol and mix by inversion. Chill the mixture for 15 minutes in a dry-ice ethanol bath or place at -70°C for at least 30 minutes. Spin down pellet, wash pellet with 90% (v/v) ethanol and redissolve in TE buffer.

8.3. Quality control

The performance of both N5000 and N5500 is tested, with the control DNA provided, using 250 pmol/µg DNA of [α-^{32}P]-labelled nucleotides, specific activity 400 Ci/mmol and 33 pmol/µg [α-^{32}P]dCTP, specific activity 3000 Ci/mmol. Incorporation of greater than 50 and 60 percent respectively is achieved after 2 hours incubation at 15°C, as assayed by thin-layer chromatography on PEI cellulose in 1.25 M KH_2P0_4, pH 3.4.

In addition, the ^{32}P-labelled DNA is analyzed on a polyacrylamide gel, under denaturing conditions, to ensure probes are of an acceptable length. Components of the kits are checked for identify by HPLC and the DNA solutions for concentration by UV spectrophotometry.

9. References

1. KELLY, R.B. *et al., J. Biol. Chem.,* **245**, pp.39-45, 1970.

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10. Related products

Radioactive probe labelling

Multiprime™ DNA labelling systems	RPN1600/1601
Megaprime™ DNA labelling systems	RPN1604-7
Rediprime™ DNA labelling kit	RPN1633/1634
3'-end labelling kit	N4020
5'-end labelling kit	RPN1509
RNA labelling system	RPN3100

Radiolabelled nucleotides

Full range of ^{32}P, ^{33}P, ^{35}S and ^{3}H labelled nucleotides for probe labelling	See catalogue for details

Non-radioactive nucleotides

Cy3 dCTP	PA53021
Cy5 dCTP	PA55021
Rapid-hyb hybridization buffer	RPN1635/1636

Hybridization membranes

Hybond range of nylon and nitrocellulose membranes	See catalogue for details

Autoradiography film

Hyperfilm™ range	See catalogue for details

A range of hybridization and detection accessories including UV cross linker, hybridization oven, film cassettes, dark room torch and film processor are all available (see catalogue for details).

Adjustable pipettes

Full range of variable volume pipettes	RPN2340-4

For further details on these products and for information on products for *in situ* hybridization and immunocytochemistry see the current GE Healthcare catalogue or contact your local GE Healthcare office.

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