user manual
hybridization

# Hybridization oven/shaker

RPN2511

Underwriters Laboratories
Inc. Listed, #E195497

Amersham Biosciences

# Hybridization Oven/Shaker
## RPN2511

### Contents



IMPORTANT: Please read the separate instructions for use before operating the Amersham Biosciences Hybridization Oven/Shaker to familiarize yourself with its electrical installation and safety precautions.

## 1.0 Introduction

One of the most widely used techniques in the molecular biology field is the immobilization of DNA and RNA onto a solid support membrane and subsequent hybridizations with a specific single stranded probe, labelled to facilitate its detection.

Using the Amersham Biosciences Hybridization Oven/Shaker ensures that the temperature and shaking/rotation frequency, and hence the stringency of hybridization and washing steps are rigidly controlled. This enables rapid and reproducible probing of nucleic acids, and proteins immobilized on nylon and nitrocellulose membranes.

The Amersham Biosciences Hybridization Oven/Shaker is a multipurpose instrument combining accurate temperature control with a choice of interchangeable hybridization modes:

- Variable speed rotisserie: holding 7 x 35 mm or 2 x 70 and 2 x 35 mm hybridization bottles.
- A variable speed platform shaker for 'box' hybridizations, depurination, denaturation and neutralization steps.

The instrument is:

- Economical: bottle hybridization minimizes probe volumes, reducing reagent volumes and enhancing signal intensity.
- Precise: stringency of hybridization and washing steps are rigidly controlled, ensuring reproducible results.
- Sensitive: validated protocols ensure optimal hybridization and washing steps, enhancing multiple reprobing when using Hybond™ membranes.
- Safe: the double-glazed polycarbonate/acrylic door offers excellent thermal insulation whilst minimizing radiation exposure.
- Convenient: small foot-print maximizes the use of limited laboratory space.

The instrument is suitable for use in conjunction with radiolabelled probes using Rapid-hyb™ buffer, non-radioactive nucleic acid labelling and detection systems such as AlkPhos Direct™, and protein labelling and detection systems including ECL™ and ECL Plus™. Some protocols for use with these applications are included in section 6.

## 2.0 Specification

| Overall dimensions | | |
|---|---|---|
| Height: | 9.5 " | (24.0 cm) |
| Depth: | 10.0 " | (25.0 cm) |
| Width: | 11.25 " | (28.5 cm) |

| Oven dimensions | | |
|---|---|---|
| Height: | 8.0 " | (20.0 cm) |
| Depth: | 9.0 " | (23.0 cm) |
| Width: | 10.0 " | (25.0 cm) |

| | |
|---|---|
| Weight: | 24 kg |
| Capacity (nominal): | 18 litres |
| Temperature range: | Ambient plus 5 °C–80 °C |
| Temperature precision: | +/- 0.5 °C |
| Temperature fluctuation: | +/- 0.1 °C (@37°C) |
| Power rating: | 250 W |
| Over temperature cut-out: | 1 °C over set temperature |
| Temperature variation: | <0.25 °C |
| Rotisserie speed: | 2–10 rev/min |
| Shaker platform speed: | 5–70 strokes/min |
| Total angle of tilt: | 4° or 10° angle |

Electrical

| Nominal Voltage/Hertz/Amp | Product code |
|---|---|
| 230 V/50 Hz/3.1 A | RPN2510 |
| 110 V/120 V/60 Hz/4 A | RPN2511 |
| 100 V/50/60 Hz | RPN2512 |

Each instrument is supplied with 1 rotisserie (RPN2514), 6 hybridization bottles (RPN2516) and an instruction manual.

Accessories

| | |
|---|---|
| Rotisserie, holds 7 x 35 mm hybridization bottles | RPN2514 |
| Rotisserie, holds 2 x 70 mm and 2 x 35 mm hybridization bottles | RPN2515 |
| Hybridization bottle, 230 x 35 mm (For rotisserie RPN2514, pack of 6) | RPN2516 |
| Hybridization bottle, 150 x 35 mm (For rotisserie RPN2514, pack of 6) | RPN2517 |
| Hybridization bottle, 230 x 70 mm (For rotisserie RPN2515, pack of 2) | RPN2518 |
| Hybridization mesh, 1 roll 21.5 cm x 10 m | RPN2519 |

## 3.0 Setting up the Hybridization Oven/Shaker

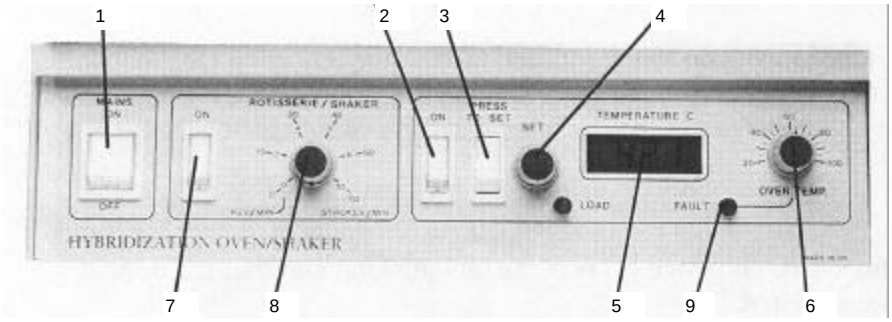
3.0.1 Remove all packaging and place the Hybridization Oven/Shaker on a level working surface, ensuring that there is sufficient room above the instrument to allow the door to be opened fully.

3.0.2 Plug the female end of the power cable into the Hybridization Oven/Shaker.

3.0.3 Connect the power cable to a suitably grounded electrical outlet. The correct operating voltage of the Hybridization Oven/Shaker is found on the product information label on the rear of the instrument.

3.0.4 Turn the Mains ON/OFF switch (1 on Fig 1) to the ON position.

3.0.5 The Amersham Biosciences Hybridization Oven/Shaker is now ready for use.




1. Mains ON/OFF switch
2. Temperature ON/OFF switch
3. Temperature SET switch
4. Temperature SET dial
5. Digital temperature display
6. Oven OVER TEMP dial
7. Rotisserie/shaker ON/OFF switch
8. Rotisserie/shaker speed selector dial
9. Oven FAULT LED

### 3.1 Fig 1. Diagram of instrument control panel

## 3.2 Setting the oven temperature

3.2.1 Press the Mains ON/OFF switch (1, see Fig 1) to the ON position.

3.2.2 Press the Temperature ON/OFF switch (2) to the ON position.

3.2.3 Press the Temperature SET switch (3), at the same time rotate the Temperature SET dial (4) until the required temperature is shown on the digital display (5).

3.2.4 Release the Temperature SET switch (3).

3.2.5 Rotate the Oven OVER TEMP dial (6) to the SET temperature +5 °C.

*NOTE: The automatic temperature cut-out operates at 1 °C above the SET temperature, to ensure that the desired temperature is maintained at all times.*

The OVER TEMP setting acts as an instrument fail safe mechanism. Should the OVER TEMP setting be reached, the red FAULT LED (9) will light up denoting a fault in the temperature control system.

3.2.6 The oven will now automatically heat up the the SET temperature.

## 3.3 Setting up the rotisserie

The rotisserie is installed in the Hybridization Oven/Shaker during transit. To use the rotisserie for bottle hybridization, the following procedure should be adopted:

3.3.1 Lift up the oven door to its fullest extent to allow complete access to the oven interior.

3.3.2 Lift the rotisserie vertically out of the oven and place on the bench.

3.3.3 Place the membranes to be hybridized in to the required number of hybridization bottles. Using the rotisserie as a stand for the bottles, place the bottles into the rotisserie, pushing them down as far as they will go.

*NOTE: Always ensure that the weight is evenly distributed on both sides of the rotisserie. Place an empty hybridization bottle into the other side of the rotisserie as a counterbalance if necessary.*

3.3.4 Place the rotisserie into the voen onto the rotation mechanism, ensuring that the serrated bands at either end of the rotisserie locate onto the steel cogs of the rotation mechanism at the rear of the oven. The plastic flanges of the rotisserie locate on to the small wheels on the oven floor. Close the oven door.

3.3.5 Ensure that the Mains ON/OFF switch (1) is in the ON position and that the desired temperature has been set (see section 4.2).

3.3.6 Turn the Rotisserie/Shaker ON/OFF switch (7) to the ON position.

3.3.7 Turn the Rotisserie/Shaker speed selector dial (8) clockwise until the desired rotation speed is reached (allowable values are 2–10 rpm).

The rotisserie will now start to rotate at the set rate.

3.3.8 When hybridization is complete turn the Rotisserie/Shaker ON/OFF switch (7) to the OFF position.

## 3.4 Setting up the platform shaker

During transit, the platform is stored vertically at the rear of the oven chamber. It can remain in this position whilst the rotisserie is in use. To use the platform shaker for 'sandwich box' hybridizations, the following procedure should be adopted:

3.4.1 Open the oven door to its fullest extent, lift the rotisserie vertically and store in a safe place.

3.4.2 Lift the platform by its handle (A, see figure 2) from its storage position, slide it forward and locate it on the rocking mechanism by placing the side pegs (8) of the platform into the retainers (C) on the side walls of the oven chamber. This action seats the nylon blocks (D) on the underside of the platform on to the pegs (E) protruding from the rocker mechanism at the rear of the oven.

3.4.3 Place the box in which the hybridization is being performed on to the shaker platform and close the oven door.

3.4.4 Ensure the Mains ON/OFF switch (1, see figure 1) is in the ON position and that the desired temperature has been set (see section 4.2).

3.4.5 Turn the Rotisserie/Shaker ON/OFF switch (7) to the ON position.

3.4.6 Rotate the Rotisserie/Shaker speed selector dial (8) clockwise, until the desired shaker speed is reached. Allowable values are 5–70 strokes per minute.

The shaker platform will now oscillate at the set rate.

3.4.7 When hybridization is complete, turn the ON/OFF switch (7) to the OFF position.

*NOTE: The shaker platform can be operated at a choice of tilt angles, 4° or 10° The larger the angle, the greater the vertical movement of the platform at the end of each stroke, and hence the greater the agitation of the contents of the box.*

This facility allows further fine-tuning of the rocking motion of the shaker platform in addition to controlling the shaker speed.

The tilt angle is controlled by the position of the pegs in the rocker mechanism, which is attached to the rear of the spill tray. To alter the tilt angle, unscrew the spill tray by rotating the nylon retaining screws anti-clockwise. Lift out the spill tray. Remove the two pegs (E in figure 2) and screw in to the appropriate position in the rocker mechanism. Figure 3 below, shows the relative positions of each hole and the tilt angle to which they correspond.

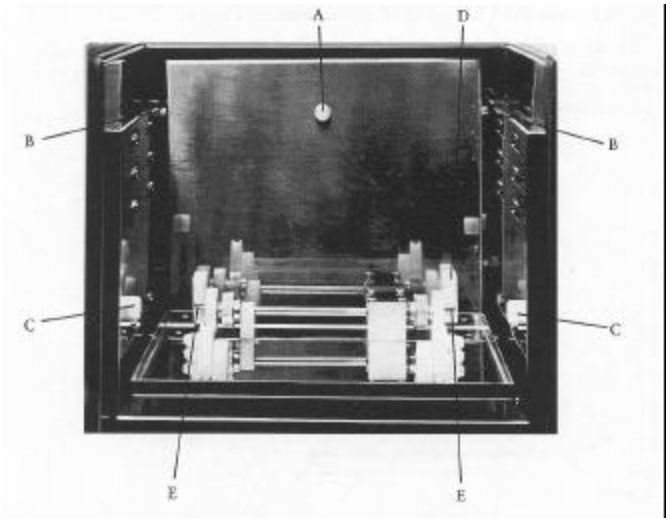



Fig 2. Hybridization oven drive components

On completion, ensure that the spill tray is repositioned correctly and screwed down securely. On starting the shaker, the drive shaft will automatically re-engage itself. This may take a few revolutions of the drive mechanism.

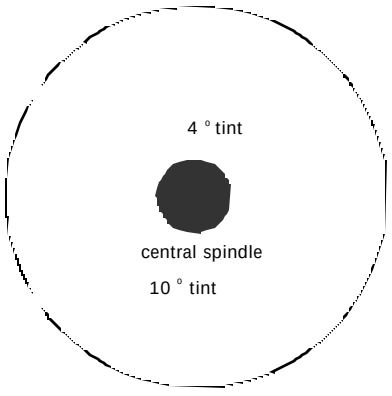



Fig 3. Adjustment of angle of tilt of platform shaker

## 4.0 Hybridization using the platform shaker

The Hybridization Oven/Shaker is compatible with the hybridization technologies available from Amersham Biosciences. These include radioactive hybridizations using Rapid hyb buffer and the range of non-radioactive systems for the labelling and detection of proteins and nucleic acids (see appendix 1).

When using the platform shaker the hybridization and washing conditions recommended in the appropriate Amersham Biosciences literature should be used. The following protocol therefore provides a guideline. For specific hybridization times and temperatures, refer to the relevant protocol booklet.

4.0.1 Prepare blots as recommended in the appropriate Hybond protocol booklet.

4.0.2 Set the oven temperature and over temperature values as described in section 4.2.

4.0.3 Place the membrane in a suitable box (or bag) and cover with sufficient prehybridization buffer to ensure that the entire surface of the membrane is covered. Recommended volume: surface area ratio is given in most protocol booklets. Seal the box (or bag) securely.

4.0.4 Place the box (or bag) on the platform, set the oscillation speed to 30 strokes/min and prehybridize for the required length of time.

4.0.5 Remove the box (or bag) from the oven and carefully add the labelled single stranded probe (denaturation may be required post labelling refer to the appropriate protocol booklet) to the prehybridization buffer.

*NOTE: Do not pipette the probe solution directly on to the membrane as this may cause localized background.*

4.0.6 Reseal the box (or bag) securely, replace it on the platform and hybridize for the required length of time.

4.0.7 Remove the membranes and place in a clean box containing the first stringency wash solution.

4.0.8 Increase the oscillation speed of the platform to 60 strokes/min. Carry out the recommended washing protocol at the appropriate temperature and for the appropriate times.

## 5.0 Hybridization using the rotisserie

Several advantages are associated with performing hybridizations in bottles, namely those of safety and economy as outlined in the introduction. However, the use of bottles for hybridizations and washing procedures requires certain adaptations to standard protocols.

### 5.1 Assembly of membranes into bottles

5.1.1 Add approximately 20 ml 2x SSC buffer into the hybridization bottle. The rotisserie acts as a convenient bottle stand.

5.1.2 Pre-wet the membrane in 2x SSC buffer and loosely roll it up.

5.1.3 Insert the rolled up membrane into the bottle and replace the cap. Ensure that the cap is screwed on securely, (hand tight plus a quarter turn). DO NOT OVERTIGHTEN, or the thread of the cap can be damaged, leading to leakages.

*NOTE: If placing several small blots into one bottle, prewet the membranes as above, and space them out along the length of the bottle with forceps.*

5.1.4 Place the bottle on a flat surface and roll it gently in the opposite direction to that which the membrane is coiled. This rolling action causes the membrane to uncoil, so lining the inner surface of the bottle.

*NOTE: The use of a mesh in bottle hybridizations to ensure uniform contact between the membrane surface and the buffer has been recommended.*

However, studies at Amersham Biosciences laboratories using a wide range of hybridization mesh technologies demonstrate a resulting loss of sensitivity due to partial absorption of the probe into the mesh.

A nylon mesh (RPN2519) is available as an optional extra, as it can facilitate easier handling of a number of blots and the more fragile nitrocellulose membranes. These handling advantages should be considered against the potential loss of sensitivity before use.

When a hybridization mesh is used in conjunction with the membrane, the following procedure should be adopted:

5.1.5 Pre-wet the mesh alongside the membrane in 2x SSC buffer and place the prewetted membrane on top of the mesh. The mesh should be slightly larger than the blot in all dimensions. Roll both up together, with the mesh on the outside of the membrane, and insert into the bottle as described above (6.1.4).

### 5.2 Hybridization

5.2.1 Set the required oven temperature and over temperature, (as detailed in section 4.2).

5.2.2 Discard the 2x SSC, used in the bottle to unroll the membrane, and replace with prehybridization buffer. Recommended volumes are provided in most Amersham Biosciences protocol booklets. Generally a minimum of 10–15 ml per 20 x 20 cm blot is advised. Seal the bottle, avoiding overtightening.

5.2.3 Place the hybridization bottle(s) into the rotisserie (as detailed in section 4.3) add counterbalance bottles if necessary. Place the rotisserie into the oven so that the bottles are rotating in the same direction as the membrane is rolled, see Figure 4 overleaf.

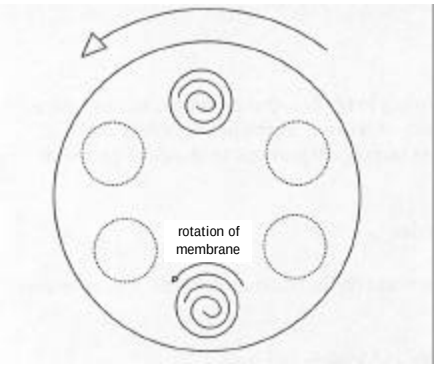



Fig 4. Rotation of rotisserie

5.2.4 Prehybridize the membrane for the specified length of time at a rotisserie speed of 4 rpm.

5.2.5 Following prehybridization, turn off the rotisserie, remove the rotisserie from the oven. Add the labelled probe to the buffer, either by removing an aliquot of the buffer, adding the probe and returning the aliquot to the bottle; or by adding the probe directly into the bottle, avoiding the membrane.

5.2.6 Hybridize for the specified length of time at a rotisserie speed of 4 rpm.

## 5.3 Membrane washing procedures

Membranes can either be removed from the hybridization bottles and washed in a box on the platform shaker (this is a more efficient procedure), or the washing procedure may be carried out in the bottles. If the platform shaker is used, follow the standard washing procedure mentioned in the appropriate protocol booklet.

If bottles are to be used it is necessary to modify the standard washing procedure.

Outlined in this section are the optimized washing protocols for radioactive hybridizations and non-radioactive based hybridizations.

### Radioactive Hybridizations

5.3.1 Carefully drain off the hybridization buffer, rinse the bottle and the membrane thoroughly with 2x SSC and discard.

5.3.2 Perform the following stringency washes in large volumes (100 ml minimum) of the following solutions, at a rotisserie speed of 8 rpm:

2 x 10 min with 2x SSC, 0.1% SDS at 65 °C

1 x 15 min with 1x SSC, 0.1% SDS at 65 °C

2 x 10 min with 0.1x SSC, 0.1% SDS at 65 °C

*NOTE: This last step is a high stringency wash and should be omitted if related sequences are to be probed.*

(More washes over the same time period for each stringency condition can improve background).

5.3.3 Remove the membrane from the bottle, drain off excess stringency wash, wrap in SaranWrap™ and autoradiograph.

### AlkPhos Direct Hybridizations

5.3.4 Drain off the hybridization buffer, rinse the bottle and the membrane thoroughly with primary wash buffer and discard.

5.3.5 Perform the following stringency washes in large volumes (100 ml minimum) of the following solutions, at a rotisserie speed of 8 rpm:

3 x 10 min with primary wash buffer solution at 55 °C

3 x 5 min with secondary wash buffer at room temperature

*NOTE: The room temperature washes can be achieved by switching off the oven and leaving the door open whilst performing the washes or by allowing the oven to cool down to room temperature before performing the final washes.*

5.3.6 Remove the membrane from the bottle and detect using the standard procedures outlined in the protocol booklet.

### Gene Images Random Prime Hybridizations

5.3.7 Drain off the hybridization buffer, rinse the bottle and the membrane thoroughly with 2x SSC and discard.

5.3.8 Perform the following stringency washes in large volumes (100 ml minimum) of the following solutions, at a rotisserie speed of 8 rpm:

2 x 10 min with 1x SSC, 0.1% SDS at 60 °C

1 x 10 min with O.1x SSC, 0.1% SDS at 60 °C

5.3.9 Remove the membrane from the bottle and detect using the standard procedures outlined in the booklet. The detection procedure may be carried out using the shaker mode of the Hybridization Oven/Shaker.

*NOTE: The room temperature washes can be achieved by switching off the oven and leaving the door open whilst performing the incubations or by allowing the oven to cool down to room temperature.*

## 6.0 Maintenance/care/cleaning of the oven/shaker

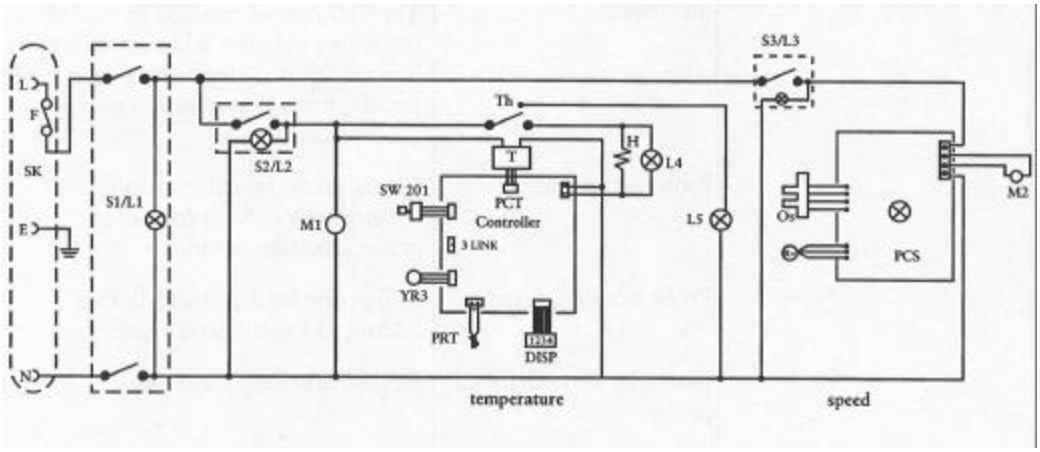
The Amersham Biosciences Hybridization Oven/Shaker is designed to provide trouble-free operation. The base of the oven and the shaker tray act as a spills tray and will contain any spillage that occurs during hybridization and washing procedures.

To ensure lasting operation the following instructions should be followed:

ALWAYS DISCONNECT THE HYBRIDIZATION OVEN/SHAKER FROM THE ELECTRICAL SUPPLY BEFORE CLEANING OR DRYING THE INSTRUMENT.

1. Any leakage from the hybridization bottles or the sandwich boxes should be cleaned up immediately. Do not allow any liquids to enter the drive mechanism.
2. Wipe away any liquids from inside and outside of the unit using soap and water with a soft cloth or sponge.
3. Do not allow chemicals to remain on unit surfaces.
4. Never clean unit with abrasive pads or cleaners.
5. Never clean unit with acetone or chloroform.

## 7.0 Wiring diagram



## 8.0 Troubleshooting guide

This section briefly summarizes some of the potential problems encountered during membrane hybridizations. More complete troubleshooting guides are supplied in the pack leaflet that accompany each product.

| Symptom | Cause | Remedy |
|---|---|---|
| Membrane curling up in hybridization bottle | Incorrect orientation of bottle in rotisserie | Ensure membrane is rolled up in the same direction as the bottle is rotating (see section 6.2) |
| High background | Probe concentration too high | Reduce probe concentration |
| | Unincorporated $^{32}$P nucleotides not removed | Remove unincorporated nucleotides eg. using Amprep™ C18 column |
| | Insufficient blocking | Use recommended hybridization buffer or extend prehybridization time |
| | Insufficient washing | Increase number of buffer changes during the washing stage or increase stringency of final wash |
| Weak signal | No transfer from gel to membrane | Load extra lanes with control DNA. Transfer can be checked by restaining gel with ethidium bromide. If large DNA fragments are detected poorly, use a depurination step (0.25 M HCl) |
| | Probe not labelled | Check probe labelling before hybridization. See protocol in probe labelling booklet |
| | Probe not denatured | Boil probe for 5 minutes before adding to hybridization buffer |
| | Low specific activity of probe | Review labelling conditions |
| | Washes too stringent | Increase buffer salt concentration and decrease temperature |
| Patchy backgrounds | Hybridization buffer or wash solution not evenly covering membrane | Increase volume of hybridization wash or solutions or use mesh (see section 6.1.4) |
| | Damaged membrane | Handle membrane carefully with forceps |
| High background around edge of membrane | Damaged membrane | Use clean scissors or a sharp scalpel to cut membrane |

## DNA Markers

| Precise Sizing | | | | | Digest of Natural DNAs | | | | Pulsed Field | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oligonucleotide sizing markers 27-2521-01 | 50 Base-pair ladder 27-4005-01 | 100 Base-pair ladder 27-4001-01 | 250 Base-pair ladder 27-4006-01 | KiloBase™ DNA marker 27-4004-01 | λDNA-Hind III Digest 27-4048-01 | X-174 RF DNA-*Hae* III Digest 27-4040-01 | X-174 RF DNA-*Hae* III Digest 27-4044-01 | λDNA-*Hind* III/X-174 RF DNA-*Hae* III Digest 27-4052-01 | λDNA-*Hind* III/X-174 RF DNA-*Hae* III Digest 27-4054-01 and DRigest III 27-4060-01 | λDNA-PFGE Markers 27-4530-01 | Yeast DNA-PFGE Markers 27-4520-01 32 |
| 32[a] | 500[a] | 2,000[a] | 2,000 | 10,000[a] | 23,130[b] | 1,057 | 1,353 | 23,130[b] | 23,130[b] | 970,000 | 1,900,000 |
| 30 | 450 | 1,900 | 1,750 | 8,000 | 9,416 | 770 | 1,078 | 9,416 | 9.416 | 921,500 | 1,640,000 |
| 28 | 400 | 1,800 | 1,500 | 6,000 | 6,557 | 612 | 872 | 6,557 | 6,557 | 973,000 | 1,120,000[d] |
| 26 | 350 | 1,700 | 1,250 | 5,000 | 4,361[b] | 495 | 603 | 4,361[b] | 4,361[b] | 824,500 | 1,110,000[d] |
| 24 | 300 | 1,600 | 1,000 | 4,000 | 2,322 | 392 | 310 | 2,322 | 2,322 | 776,000 | 945,000 |
| 22 | 250 | 1,500 | 750 | 3,000 | 2,027 | 345 | 281 | 2,027 | 2,027 | 727,500 | 915,000 |
| 20 | 200 | 1,400 | 500 | 2,500 | 564 | 341 | 271 | 1,057 | 1,353 | 679,000 | 815,000 |
| 18 | 150 | 1,300 | 250 | 2,000 | 125[c] | 335 | 234 | 770 | 1,078 | 630,500 | 785,000 |
| 16 | 100 | 1,200 | | 1,500 | | 297 | 194 | 612 | 872 | 582,000 | 745,000 |
| 14 | 50 | 1,100 | | 1,000 | | 291 | 118 | 564[c] | 603 | 533,500 | 680,000 |
| 12 | | 1,000 | | 500 | | 210 | 72 | 495 | 564[c] | 485,000 | 610,000 |
| 10 | | 900 | | | | 162 | | 392 | 310 | 436,500 | 555,000 |
| 8 | | 800 | | | | 79 | | 345 | 281 | 388,000 | 450,000 |
| | | 792 | | | | | | 341 | 271 | 339,500 | 375,000. |
| | | 700 | | | | | | 335 | 234 | 291,000 | 295,000 |
| | | 600 | | | | | | 297 | 194 | 242,500 | 225,000 |
| | | 500 | | | | | | 291 | 125[c] | 194,000 | |
| | | 400 | | | | | | 210 | 118 | 145,500 | |
| | | 300 | | | | | | 162 | 72 | 97,000 | |
| | | 200 | | | | | | 125[c] | | 48,500 | |
| | | 100 | | | | | | 79 | | | |

a not necessarily the largest size fragment possible, only the largest that is readily distinguishable
b cos ends are located on these bands
c very faint band
d chromosomes VII and XV run as a single band
e may not always be visible

| Marker code number | Recommended gel | Loading amount (µg/lane) | Heat before loading |
|---|---|---|---|
| 27-2521-01 | 20% polyacrylamide/7 M urea | 3 µl radiolabelled marker/lane | 90 °C for 3 min |
| 27-4005-01 | 2% agarose/6% polyacrylamide | 2.0 | No |
| 27-4001-01 | 1.5% agarose | 2.0 | No |
| 27-4006-01 | 1% agarose | 2.0 | No |
| 27-4004-01 | 0.8% agarose | 0.5 | No |
| 27-4048-01 | 1% agarose | 0.5 | 60–65 °C for 2 min |
| 27-4040-01 | 1% agarose | 0.5 | No |
| 27-4044-01 | 1% agarose | 0.5 | No |
| 27-4052-01 | 1% agarose | 0.5–1.0 | 60–65 °C for 2 min |
| 27-4054-01 | 1% agarose | 0.5–1.0 | 60–65 °C for 2 min |
| 27-4060-01 | 1% agarose | 0.5–1.0 | 60–65 °C for 2 min |

| Size | Pack size | Hybond N+ | Hybond XL | Hybond N | Hybond NX | Hybond Nfp | Hybond ECL | Hybond C Extra | Hybond P |
|---|---|---|---|---|---|---|---|---|---|
| 82 mm | 50 discs | RPN82B | RPN82S | RPN82N | RPN82T | | RPN82D | RPN82E | |
| 87 mm | 50 discs | RPN87B | RPN83S | RPN87N | RPN87T | | | | |
| 132 mm | 50 discs | RPN132B | RPN132S | RPN132N | RPN132T | | RPN132D | | |
| 137 mm | 50 discs | RPN137B | RPN137S | RPN137N | RPN137T | | RPN137D | RPN137E | |
| 11.9 x 7.8 cm | 50 sheets | RPN119B | RPN119S | RPN119N | RPN119T | | | | |
| 12 x 10 cm | 20 sheets | RPN1210B | RPN1210S | RPN1210N | RPN1210T | | | | |
| 15 x 10 cm | 20 sheets | RPN1510B | RPN1510S | RPN1510N | RPN1510T | | | | |
| 15 x 20 cm | 10 sheets | RPN1520B | RPN1520S | RPN1520N | RPN1520T | | RPN1520D | | |
| 20 x 20 cm | 10 sheets | RPN2020B | RPN2020S | RPN2020N | RPN2020T | | RPN2020D | RPN2020E | RPN2020F |
| 22.2 x 22.2 cm | 10 sheets | RPN2222B | RPN2222S | RPN2222N | RPN2222T | | | | |
| 30 x 50 cm | 5 sheets | RPN3050B | RPN3050S | RPN3050N | RPN3050T | | | RPN3050E | |
| 20 cm x 3 m | 1 roll | RPN203B | RPN203S | RPN203N | RPN203T | RPN203X | RPN203D | RPN203E | RPN303F |
| 30 m x 3 m | 1 roll | RPN303B | RPN303S | RPN303N | RPN303T | | | | |
| 82 mm | 50 gridded discs | RPN1782B | | | | | | | |
| 87 mm | 50 gridded discs | RPN1787B | | | | | | | |
| 132 mm | 50 gridded discs | RPN1732B | | | | | | | |
| 137 mm | 50 discs | RPN1737B | | | | | | | |
| 22.2 x 22.2 cm | 50 sheets | RPN2250B | | | | | | | |
| 22.5 x 22.5 cm | 50 sheets | RPN225B | | | | | | | |
| | 50 sheets | RPN1576B | | | | | | | |
| 6 x 8 cm | 50 sheets | | | | | | RPN68D | | |
| 7 x 8 cm | 50 sheets | | | | | | RPN78D | | |
| 16 x 14 cm | 15 sheets | | | | | | | | RPN1416F |
| 9 x 10 cm | 10 sheets | | | | | | RPN910D | | |
| 15 x 15 cm | 10 sheets | | | | | | RPN1515D | | |
| 10 x 10 cm | 10 sheets | | | | | | RPN1010D | | |
| 30 cm x 3 m | 1 roll* | | | | | | RPN3032D | | |

*0.2 µm pack size

## Appendix 3. Radioactive labelling systems

| Labelling system | Technology | Nucleotide | Amount of template | Labelling time | Probe specific activity (dpm/µg) | Recommended application |
|---|---|---|---|---|---|---|
| Rediprime II™ | Random-Prime | dCTP only | 25 ng | 10 min | $2 \times 10^9$ | membrane hybridization |
| Ready-To-Go™ DNA labelling beads | Random-Prime | dCTP only | 10 ng–1 µg | 5 min | $2 \times 10^9$ | membrane hybridization |
| Megaprime | Random-Prime | any dNTP | 25 ng | 10 min | $2 \times 10^9$ | membrane hybridization |
| Nick™ translation | Nick translation | any dNTP | 1 µg | 2–3 hours | $2 \times 10^9$ | production of large amounts of probe |
| RNA labelling | SP6/T7 RNA polymerase | UTP | 1 µg | 1–2 hours | $2 \times 10^9$ | *in situ* hybridization |
| 5'-end labelling | T4 polynucleotide kinase | dATP | 10 pmol ends | 1 hour | $5 \times 10^8$ | membrane hybridization *in situ* hybridization |
| 3'-end labelling | Terminal deoxy-nucleotidyl transferase | any dNTP | 10 pmol ends | 30–60 min | $5 \times 10^8$ | membrane hybridization *in situ* hybridization |

# Appendix 4. Radioactive nucleotides

| Compound | Concentration | Specific activity | | Redivue product code | Standard product code | Pack size (see key) |
|---|---|---|---|---|---|---|
| | | TBq/mmol | Ci/mmol | | | |
| $^{32}$P | | | | | | |
| **[a-$^{32}$P]dATP** | 10 mCi/ml | ~220 | ~6000 | AA0074 | PB10474 (a) | 1, 2 & 3 |
| | | ~110 | ~3000 | AA0004 | PB10204 (a) | 1, 2 & 3 |
| | | ~30 | ~800 | AA0084 | PB10384 (a) | 1, 2 & 3 |
| | | ~15 | ~400 | AA0064 | PB10164 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]dCTP** | 10 mCi/ml | ~220 | ~6000 | AA0075 | PB10475 (a) | 1, 2 & 3 |
| | | ~110 | ~3000 | AA0005 | PB10205 (a) | 1, 2 & 3 |
| | | ~30 | ~800 | AA0085 | PB10385 (a) | 1, 2 & 3 |
| | | ~15 | ~400 | AA0065 | PB10165 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]dGTP** | 10 mCi/ml | ~110 | ~3000 | AA0006 | PB10206 (a) | 1, 2 & 3 |
| | | ~30 | ~800 | AA0086 | PB10386 (a) | 1, 2 & 3 |
| | | ~15 | ~400 | AA0066 | PB10166 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]dTTP** | 10 mCi/ml | ~110 | ~3000 | AA0007 | PB10207 (a) | 1, 2 & 3 |
| | | ~30 | ~800 | AA0087 | PB10387 (a) | 1, 2 & 3 |
| | | ~15 | ~400 | AA0067 | PB10167 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]ATP** | 10 mCi/ml | ~110 | ~3000 | | PB10200 (a) | 1, 2 & 3 |
| | | ~15 | ~400 | | PB10160 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]CTP** | 10 mCi/ml | ~110 | ~3000 | | PB10202 (a) | 1, 2 & 3 |
| | 20 mCi/ml | ~30 | ~800 | | PB20382 (b) | 1, 2 & 3 |
| | | ~15 | ~400 | | PB10162 (a) | 1, 2 & 3 |
| | 40 mCi/ml | ~30 | ~800 | | PB40382 (e) | 3 |
| **[a-$^{32}$P]GTP** | 10 mCi/ml | ~110 | ~3000 | | PB10201 (a) | 1, 2 & 3 |
| | | ~15 | ~400 | | PB10161 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]UTP** | 10 mCi/ml | ~110 | ~3000 | AA0003 | PB10203 (a) | 1, 2 & 3 |
| | 20 mCi/ml | ~30 | ~800 | | PB20383 (b) | 1, 2 & 3 |
| | | ~15 | ~400 | | PB10163 (a) | 1, 2 & 3 |
| | 40 mCi/ml | ~30 | ~800 | | PB40383 (e) | 3 |
| **[g-$^{32}$P]ATP** | 10 mCi/ml | >185 | >5000 | AA0018 | PB10218 (a) | 1, 2 & 3 |
| | 2 mCi/ml | >185 | >5000 | | PB218 (c) | 1, 2 & 3 |
| | | ~110 | ~3000 | AA0068 | PB10168 (a) | 1, 2 & 3 |
| | | ~110 | ~3000 | | PB168 (c) | 1, 2 & 3 |
| | 10 mCi/ml | ~1.11 | ~30 | | PB10132 (a) | 1, 2 & 3 |
| | 2 mCi/ml | ~0.11 | ~3.0 | | PB108 (c) | 1, 2 & 3 |
| | 2 mCi/ml | ~0.11 | ~3.0 | | PB170 (d) | 1, 2 & 3 |
| **[g-$^{32}$P]GTP** | 10 mCi/ml | >185 | >5000 | | PB10244 (a) | 1 & 3 |
| **[a-$^{32}$P]ddATP** | 10 mCi/ml | >185 | >5000 | | PB10235 (a) | 1 & 3 |
| | | ~110 | ~3000 | | PB10233 (a) | 1 & 3 |
| **[a-$^{32}$P]ATP** | 2 mCi/ml | ~1.11 | ~30 | | PB171 (d) | 1, 2 & 3 |
| **[$^{32}$P]pCp** | 10 mCi/ml | ~110 | ~3000 | | PB10208 (a) | 1, 2 & 3 |
| **[$^{32}$P]NAD** | 10 mCi/ml | ~37 | ~1000 | | PB10282 (g) | 1 & 3 |
| $^{33}$P | | | | | | |
| **[g-$^{32}$P]ATP** | 10 mCi/ml | 37–110 | 1000–3000 | AH9968 | BF1000 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]dATP** | 10 mCi/ml | 37–110 | 1000–3000 | AH9904 | BF1001 (a) | 1, 2 & 3 |
| **[a-$^{32}$P]UTP** | 20 mCi/ml | 37–110 | 1000–3000 | | BF1002 (a) | 1 |
| $^{35}$S | | | | | | |
| **[$^{35}$S]dATPaS** | 10 mCi/ml | >37 | >1000 | AG1000 | SJ1304 | 1 & 3 |
| | | ~22 | ~600 | AG1001 | SJ304 | 1 & 3 |
| | | ~15 | ~400 | AG1002 | SJ264 | 1 & 3 |
| **[$^{35}$S]dCTPaS** | 10 mCi/ml | >37 | >1000 | | SJ1305 | 1 & 3 |
| | | ~22 | >600 | | SJ305 | |
| | | ~15 | ~400 | | SJ265 | |
| **[$^{35}$S]dATPaS** | 10 mCi/ml | >37 | >1000 | | SJ1334 (h) | 1 & 3 |

| Compound | Concentration | Specific activity | | Redivue | Standard | Pack size |
|---|---|---|---|---|---|---|
| | | TBq/mmol | Ci/mmol | product code | product code | (see key) |
| **[$^{35}$S]ATP$\alpha$S** | | >37 | >1000 | | SJ1300 | |
| | | ~22 | ~600 | | SJ300 | |
| | | ~15 | ~400 | | SJ260 | |
| **[$^{35}$S]CTP$\alpha$S** | 10 mCi/ml | >37 | >1000 | | SJ1302 | 1 & 3 |
| | 40 mCi/ml | ~30 | ~800 | | SJ40382 (f) | 3 |
| **[$^{35}$S]UTP$\alpha$S** | 10 mCi/ml | >37 | >1000 | | SJ1303 | 1 & 3 |
| | 20 mCi/ml | >37 | >1000 | | SJ603 (i) | 1 & 3 |
| | 10 mCi/ml | ~15 | ~400 | | SJ263 | 1 & 3 |
| | 40 mCi/ml | ~30 | ~800 | | SJ40383 (f) | 3 |
| **[$^{32}$S]ATP$\gamma$S** | 10 mCi/ml | >37 | >1000 | | SJ1318 | 1 & 3 |
| | 10 mCi/ml | ~22 | ~600 | | SJ318 | 1 |

*NOTE: Redivue $^{32}$P- and $^{33}$P-nucleotides contain dye and stabilizer in formulation A. All $^{35}$S-nucleotides are supplied in stabilized aqueous solution (containing 20 mM DTT) at 370 MBq/ml, 10 mCi/ml except where stated.*

Formulation decoder

| | |
|---|---|
| A | Stabilized aqueous solution (containing 5 mM 2-mercaptoethanol) at 370 MBq/ml, 10 mCi/ml |
| B | Stabilized aqueous solution (containing 5 mM 2-mercaptoethanol) at 740 MBq/ml (SP6/T7 grade) |
| C | Ethanol: water (1.1) now supplied in the CDC container at 74 MBq/ml, 2 mCi/ml |
| D | Aqueous solution at 74 MBq/ml, 2 mCi/ml |
| E | Stabilized aqueous solution at 1.5 GBq/ml, 40 mCi/ml (SP6/T7 grade) |
| F | SP6/T7 Grade supplied at 1.5 GBq/ml, 40 mCi/ml |
| G | Stabilized aqueous solution pH6.0 |
| H | Contains no DTT (in situ grade) |
| I | Stabilized aqueous solution (containing 20 mM DTT at 740 MBq/ml, 20 mCi/ml (SP6/T7 grade) |

Pack size key

1: 9.25 MBq, 250 µCi
2: 18.5 MBq, 500 µCi
3: 37 MBq, 1 mCi

## Appendix 5. Non-radioactive labelling and detection systems

| Labelling and detection system | Sensitivity | Time from hybridization to detection | Duration of light output | Strip before re-probing | Quantification | Recommended application |
|---|---|---|---|---|---|---|
| AlkPhos Direct | 0.06 pg | 1 hour | 5 days | yes | no | Single copy Southern and Northerns |
| ECL Direct | 0.5 pg | 1 hour | 1–2 hours | no | no | High target applications eg. colony/plaques |
| Gene Images™ Random Prime | 0.1 pg | 3 hours | 5 days | yes | no | High sensitivity Northerns |
| Gene Images 3'-end labelling with CDP-Star | 0.1 pg | 3 hours | 5 days | yes | no | Oligo screening with stringency control |
| Gene Images 3'-end labelling with ECF™ | 120 pg | 3 hours | 1–2 days | yes | yes | Quantification |
| ECL Random-Prime | 0.5 pg | 3 hours | 1–2 hours | yes | no | Medium target Southern with DNA probes |
| ECL 3'-end labelling | 0.2 pg | 3 hours | 1–2 hours | yes | no | Medium to high target Southern with oligo probes |
| ECF Random-Prime | 0.25 pg | 3 hours | 1–2 days | yes | yes | Quantification |

# Appendix 6. Products for autoradiography and chemiluminscent detection

## Hyperfilm – high performance autoradiography films

| Size | Pack size | Hyperfilm MP: multipurpose film autoradiography film | Hyperfilm ECL: for use with enhanced chemiluminescence |
|---|---|---|---|
| 18 x 24 cm | 25 sheets | RPN6K | RPN2103K |
| 18 x 43 cm | 25 sheets | RPN36K | – |
| 30 x 40 cm | 25 sheets | RPN7K | RPN2104K |
| 35 x 43 cm | 25 sheets | RPN8K | – |
| 35 x 43 cm | 75 sheets | RPN30K | – |
| 20 cm x 25 m | 1 roll | RPN34K | – |
| 18 x 24 inches | 75 sheets | RPN1675K | RPN3103K |
| 5 x 7 inches | 25 sheets | RPN1676K | RPN1674K |
| 8 x 10 inches | 25 sheets | RPN1677K | RPN2114K |
| 8 x 10 inches | 75 sheets | RPN1678K | RPN3114K |
| 10 x 12 inches | 25 sheets | – | RPN1681K |

Hypercassette™ – cassettes for autoradiography and light detection

| Size | Code (standard) | Code (deep) |
|---|---|---|
| 18 x 24 cm | RPN11642 | RPN1628 |
| 24 x 30 cm | RPN11643 | |
| 30 x 40 cm | RPN11644 | RPN1627 |
| 35 x 43 cm | RPN11645 | |
| 18 x 43 cm | RPN11646 | |
| 20 x 40 cm | RPN11647 | |
| 5 x 7 inches | RPN11648 | |
| 8 x 10 inches | RPN11649 | RPN1629 |
| 10 x 12 inches | RPN11650 | |

Hyperscreen™ – intensifying screens for $^{32}P$ and $^{125}I$ autoradiography

| Size | Code | Quantity |
|---|---|---|
| 18 x 24 cm | RPN1662 | 1 pair |
| 24 x 30 cm | RPN1663 | 1 pair |
| 30 x 40 cm | RPN1664 | 1 pair |
| 35 x 43 cm | RPN1665 | 1 pair |
| 18 x 43 cm | RPN1666 | 1 pair |
| 20 x 40 cm | RPN1667 | 1 pair |
| 5 x 7 inches | RPN1668 | 1 pair |
| 8 x 10 inches | RPN1669 | 1 pair |
| 10 x 12 inches | RPN1670 | 1 pair |

Miscellaneous

| Product name | Code |
|---|---|
| Hypertorch™<br>Battery powered LED darkroom torch, pack of 3 | RPN1620 |
| Sensitize™<br>Optimized preflash system | RPN2051 |
| TrackerTape™<br>An adhesive, waterproof tape that phosphoresces to give a permanent written image on film. For use with radioactive or chemiluminescent emissions | RPN2050 |

## Appendix 7. Hybridization products

| Product name | Pack size | Product code |
|---|---|---|
| Rapid-Hyb buffer | | |
| Rate enhanced hybridization buffer | 125 ml | RPN1635 |
| for use with radiolabelled nucleic acid probes | 500 m | RPN1636 |
| Hybridization buffer tablets | 100 tablets | RPN131 |
| Each tablet makes 10 ml of hybridization buffer for use with any nucleic acid probe | | |

## Appendix 8. Radiation safety products

### For the safe handling and storage of $^{32}P$

| | Product code | Pack description |
|---|---|---|
| Beta shielding is manufactured from 10 mm heavy gauge, optical quality acrylic which effectively absorbs $^{32}P$ beta particles | | |
| Beta starter pack – comprehensive shielding and containment package for $^{32}P$ users | RPN2052 | 1 pack |
| Beta safety screen, 15 ° – ideal when working seated at the bench | RPN1536 | 1 screen |
| Beta safety screen, 45 ° – when working standing up | RPN1537 | 1 screen |
| Beta side/rear screen – use vertically or horizontally to protect your colleagues | RPN2034 | 1 screen |
| Beta workbox – for the storage of low level radioactive working | RPN1539 | 1 box |
| Beta workbox insert – for the storage of 32 microcentrifuge tubes | RPN1540 | 1 insert |
| Beta safe storage box – holds 3 tube racks or workstations | RPN1541 | 1 box |
| Beta work tank – beta workstation, gives 360 ° protection from beta radiation | RPN2033 | 1 worktank |
| Beta heavy duty tube rack – 35 mm acrylic with 4 tube holes | RPN1543 | 1 rack |
| Beta waste safe – safe short-term storage of low activity waste awaiting disposal 400 x 240 x 220 mm 10 mm acrylic | RPN1532 | 1 safe |
| Beta midi waste safe – designed to hold standard 'sharps' 315 x 225 x 235 mm | RPN2038 | 1 safe |
| Beta mini waste safe – compatible with the Beta work tank 150 x 120 x 100 mm | RPN2039 | 1 safe |
| Beta tip safe – mini waste safe with hinged lid for safe pipette tip disposal 150 x 150 x 150 mm | RPN2081 | 1 safe |
| Beta tip safe plastic bags – disposable draw-string plastic bags | RPN2083 | pack of 25 |
| Beta pipette guard – designed for use with Gilson Pipetman™ range of pipettes | | |
| for use with the P20/100 size | RPN1544 | 1 guard |
| for use with the P200 size | RPN1545 | 1 guard |
| for use with the P1000 size | RPN1546 | 1 guard |
| set of beta pipette guards (RPN1544, RPN1545, RPN1546) | RPN1556 | 3 guards |

### Radiation safety accessories

| | | |
|---|---|---|
| Safety tray – a defined working area for bench radioactive work | | |
| 685 x 455 mm | RPN1534 | 1 tray |
| 685 x 455 mm (white**) | RPN1533 | 1 tray |
| 685 x 535 mm | RPN2042 | 1 tray |
| 530 x 330 mm | RPN2043 | 1 tray |
| 1120 x 535 mm | RPN2063 | 1 tray |
| 565 x 535 mm | RPN2083 | 1 tray |
| 455 x 255mm | RPN2093 | 1 tray |

**all other safety trays are yellow

| | Product code | Pack description |
|---|---|---|
| Kodak™ APET safety tray liners – similar chemical resistance to above liners, but with none of the disposal problems associated with plastics such as PVC. | | |
| to match RPN1533/34 | RPN1528 | pack of 25 |
| to match RPN2042 | RPN2048 | pack of 25 |
| to match RPN2043 | RPN2058 | pack of 25 |
| to match RPN2063 | RPN2068 | pack of 25 |
| to match RPN2083 | RPN2088 | pack of 25 |
| to match RPN2093 | RPN2098 | pack of 25 |
| Work box insert – compatible with work boxes and safe storage boxes | | |
| Work box insert, 1.5 ml tubes | RPN1540 | 1 insert |
| Work box insert, 2.0 ml tubes | RPN2035 | 1 insert |
| Work box insert, 0.5 ml tubes | RPN2036 | 1 insert |
| Microcentrifuge tube rack – 'S' shaped rack with lifting points to allow easy removal from boxes | | |
| Microcentrifuge tube rack, 1.5 ml tubes | RPN1542 | 1 rack |
| Microcentrifuge tube rack, 0.5 ml tubes | RPN2037 | 1 rack |
| Redivial station – designed to hold 2 Redivials and 16 microcentrifuge tubes | RPN1585 | 1 workstation |
| CDC storage box – lockable storage box for 4 Amersham Biosciences CDS's | RPN2032 | 1 box |
| Radioactive spills kit – wall mountable kit containing materials to clear up small radioactive spills | RPN2030 | 1 kit |
| Radioactive spills refill pack – refill pack for Radioactive spills RPN2030 | RPN2031 | 1 pack |

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