THERMO ELECTRON CORPORATION

Hybaid Shake ‘n’ Stack

USER INSTRUCTION MANUAL

Thermo Electron Corporation
Bioscience Technologies Division

USA and rest of world: (001) 866 984 3766
UK and Europe: +44 (0) 1256 817282

Internet: www.thermo.com/molecularbiology
Email: mol.biol@thermo.com

STOCK CODE:
HB-ST-MAN

FS31999

HYBAID SHAKE ‘N’ STACK
Warranty

Thermo Electron Corporation guarantees that the Hybaid Shake ‘n’ Stack you have received has been thoroughly tested and meets its published specification. This guarantee is valid for 12 months only if the product and functions have been used according to the instruction manual.

This warranty period can be extended to a total of **24 months (free of charge)** by completing the warranty registration card supplied with the instrument, also available online at **www.thermo.com/warrantylog.**

No liability is accepted for loss or damage arising from the incorrect use of the Hybaid Shake ‘n’ Stack. Thermo's liability is limited to the repair or replacement of the unit or refund of the purchase price at Thermo's option. Thermo is not liable for any consequential damages.

Thermo reserves the right to alter the specification of the Hybaid Shake ‘n’ Stack without prior notice. This will enable us to implement developments as soon as they arise.

The Hybaid Shake ‘n’ Stack is for research use only.

The Hybaid Shake ‘n’ Stack has not been designed for use with hazardous or volatile chemicals with low flash points such as dimethylformamide or chloroform. Thermo cannot accept responsibility for damages arising from such use. If in any doubt, contact Thermo’s product specialist prior to use.

Read the Instruction Manual carefully before using the Hybaid Shake ‘n’ Stack to ensure that you obtain the best possible results from the Oven.

NB: If the Oven is not used as specified in this Manual, the protection provided by the equipment may be impaired.

We are always interested to receive feedback on our products and services. Please e-mail your comments to **quality@thermohybaid.com** or fill in our online customer satisfaction survey at: **www.thermo.com/css**

HYBAID SHAKE 'N' STACK
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HYBRIDISATION BOTTLE SAFETY: It is important to follow correct safety procedures when using Hybridisation Bottles. Please see Appendix II for details.

CHAPTER 1
HYBAID SHAKE 'N' STACK
Introduction

The Thermo system of Hybridising in bottles is considered by many molecular biologists to be the best method for conducting Hybridisations with Southern, Northern, Dot, Slot or Colony Blots. Additional information, including detailed protocols, is contained in the Thermo Hybridisation Guide.

Hybridising in bottles means that probe volumes may be significantly reduced compared to experiments performed in conventional systems, and the continual movement of the probe across the surface of the membrane results in very efficient hybridisation reactions.

Protection from exposure to radiation is provided by the heavy walled borosilicate glass bottles. The Hybridisation Oven itself provides additional shielding. In the event of a spillage within the Oven, the stainless steel drip tray will contain up to 200mls of liquid.

NOTE: If the Hybaid Shake 'n' Stack is not used as specified in this manual, the protection provided by the equipment may be impaired.

Oven Temperature Monitoring

All Ovens are calibrated by matching the thermistor temperature read-out, seen on the LCD display, to that actually measured in fluid contained within a Hybridisation Bottle as it rotates. The thermometer used inside the calibration bottle is traceable to national standards.

This calibration method ensures all experiments carried out using the rotisserie meet our temperature accuracy specification of ±1°C and indeed, due to the rotisserie action, uniformity within the bottle is ±0.25°C. However, it is important to note that the temperature inside the oven chamber will vary from point to point and therefore, the temperature of objects/vessels, which are not placed in the rotisserie, will vary from that which is displayed. Please refer to page 4 for recommendations on temperature setting when using the shaking platforms.

The Hybaid Shake 'n' Stack is designed to be used with Thermo's hybridisation bottle. These high quality leak-proof containers can be used at temperatures up to 70°C *(for catalogue numbers see Chapter 8).*

Bottles should not be used above 70°C without taking the precaution of releasing the pressure formed by the release of gases from solutions as they are warmed. The pressure should be relieved by simply loosening and then retightening the bottle cap at approximately 60°C.

Remember to always wear gloves when handling the bottle and to use the bottle gripper provided *(see page 29 - Appendix II - for full instructions on Bottle Care).*

Finally, the Hybaid Shake 'n' Stack Oven has been designed for reliability and for ease of maintenance. Hence, the rotisserie, shaking platform and drip tray can be easily removed for cleaning.

Safety Precautions

Supply

Power rating:	250W
Frequency:	50/60Hz
Fuse rating:	110/120V: 6.3A type T
	220/240V: 1.6A type T
Ingress Protection Rating	IP22

Rotisserie speed control PCB fuse: 500mA (Note this item should not be serviced by the customer)

Working Environment: 4°C to 35°C

Care should be taken when lifting the Oven. It is advisable that help is sought when removing an Oven from its packaging and when stacking Ovens for use.

During operation, caution should be taken with moving parts that are accessible when the Oven door is open.

Thermo offers full service and technical support for all its products.

International Sales, Service & Technical Support

UK and Europe: **ukservice@thermols.com**	+44 (0) 1256 817282
USA and rest of the world **hybaidservice@thermohybaid.com**	+1 866 984 3766

Alternatively, contact your local authorised distributor.

CHAPTER 2
HYBAID SHAKE 'N' STACK
Unpacking & Installation

Hybaid Shake 'n' Stack HBSNSRS/110/220
Hybaid Shake 'n' Stack Oven
4 Levelling Feet
Shaking Tray
Shaking Tray Drive Arm Assembly
Shaking Tray Bracket
Power Cable
Drip Tray
Instruction Manual

Hybaid Shake 'n' Stack HBSNSR/110/220
Hybaid Shake 'n' Stack Oven
4 Levelling Feet
Power Cable
Drip Tray
Instruction Manual

If any item is missing or damaged, contact Thermo, or Thermo's authorised distributor.

If it is intended to use the Oven with radioactive isotopes, it must be located in a designated Radiation Area. Radiation safety procedures must be followed at all times. In the event of a spillage refer to Chapter 6 for guidance on cleaning and decontamination.

All Hybridisation Oven standard rotisseries are designed to accept the Thermo large (HBOVBL), medium (HBOVBM) or small (HBOVBS) Hybridisation Bottles.

Once unpacked, attach levelling feet to base, and site on a flat surface. Level the Oven by adjusting the height of the levelling feet. Once levelled the Oven is ready for use. If adding to a stack of Hybaid Shake 'n' Stack Ovens (maximum number is 3), attach feet and adjust height, then position feet in the 4 indents on the top of the supporting Hybaid Shake 'n' Stack Oven. Some additional adjustment of the feet may be necessary until the Ovens sit in a stable position.

NOTE: Hybaid Shake 'n' Stack Ovens have been designed to stack 3 units high. No attempt should be made to stack higher than this.

Connect each individual Oven to the mains supply using only the removable mains lead supplied. The mains lead should be fitted with a 10A fuse for both 110/120V and 220/240V regions. The Oven is earthed via the mains lead and should only be connected to an earthed supply. (*See Technical Specifications Chapter 8*).

Operation

The two switches found on the control panel operate the power and the rotisserie drive. The power switch is marked with a "1" and a "0". By switching to the "1" position, the mains power is connected to the Oven and a green section of the switch is exposed to indicate that the Oven is "ON". The switch to the right operates the rotisserie/shaker. The rotisserie/shaker switch cannot be operated without first turning on the power switch. To the left of the rotisserie/shaker switch is a dial, which can be used to adjust the rotisserie speed from 5-15rpm and the shaker speed from 4-10spm.

Instructions on how to set up the shaking platform are given in Chapter 4.

Setting the Required Temperature

The temperature controller has 3 buttons, 1 on the left and 2 on the right of the temperature display.

When the power is switched on, the LED will light up and the controller will go through a number of self-check screens, which are completed within 10 seconds. The display will then show the temperature of the Oven.

To set the temperature, press button A whilst pressing the UP or DOWN arrow button (B & C) until the required operating temperature is displayed. Once the required temperature is displayed, release button A. The Oven then begins to heat to the required temperature.

1. To increase/decrease the set point temperature press button A. This will display the current set temperature.

2. Keep button A depressed and simultaneously press down B or C to increase/decrease the set point temperature.

3. When the required set point is reached, release both buttons. The controller will now continuously display the actual temperature of the Oven.

The warm up time for the Oven varies according to the loading and the ambient temperature. When first switching on the Oven (no bottles installed) allow approximately one hour for the temperature to stabilise. Solutions should be pre-warmed to the required temperature to minimise sample stabilisation time.

For ease of use and reproducibility when hybridising in bottles, the temperature display is calibrated to show internal bottle temperature as opposed to oven air temperature. When the shaking platform is used, allowance must be made for the difference between the temperature shown on the display and the temperature achieved within a liquid filled vessel on the shaker. Raising the Oven set temperature by 2°C will ensure the desired temperature is achieved within a liquid filled vessel (e.g. sandwich box) on the shaker. For instance, if 42°C is required, then the temperature should be set to 44°C.

The lowest target temperature which the Oven can achieve is 8°C above ambient temperature. Avoid placing the unit in direct sunlight, or in any area where it is likely to be exposed to hazardous or volatile chemicals *(refer to Warranty Section).*

When siting the Hybridisation Oven, ensure a gap of at least 5cm is maintained between the sides of the Oven and any other equipment or object.

The Oven may be sited in a fume cabinet or similar chamber provided the cabinet is not used to contain hazardous or volatile chemicals with low flash points.

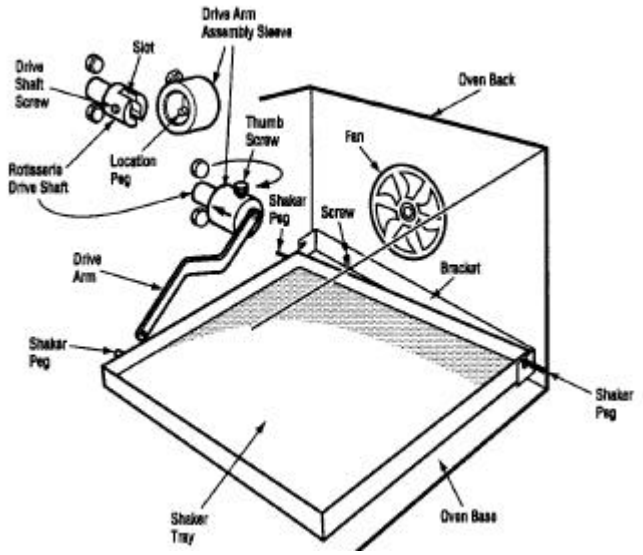
Full details of Technical Specifications are given in Chapter 8.

PLEASE NOTE: If the Hybaid Shake 'n' Stack Oven is not used as specified in this manual, the protection provided by the equipment may be impaired.

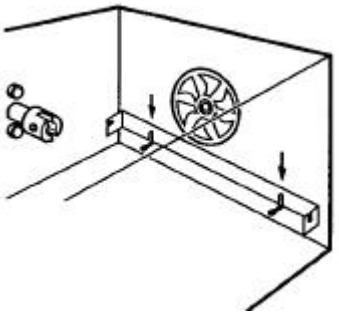
CHAPTER 3
HYBAID SHAKE 'N' STACK
Setting up the Shaking Platform

Fitting the Shaking Platform

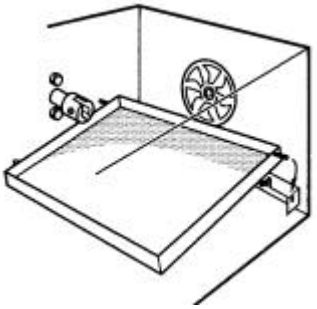
Figure 3.1: *Shaking Tray Components*



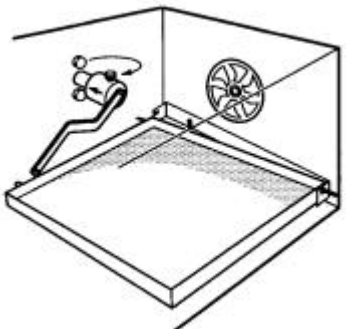
1. Loosen existing screws at the bottom of the Oven back sufficiently to allow slots in bracket to slide down onto screw shafts. Retighten screws to secure back plate.



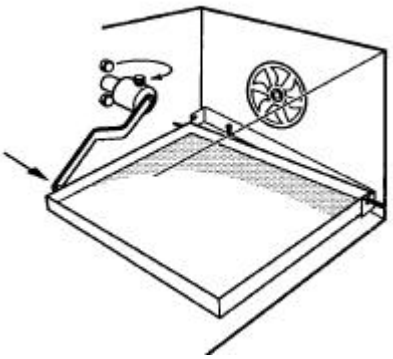
2. Fit the rear right hand peg of tray into vertical slot at right of bracket, allowing tray to temporarily rest on Oven base.



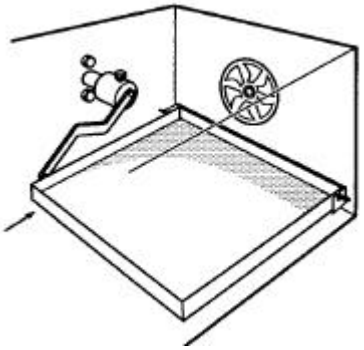
3. Slide drive arm assembly sleeve onto rotisserie drive shaft, aligning location peg within drive arm sleeve into either slot on drive shaft, ensuring the sleeve is pushed fully onto shaft. Tighten using thumb screw.



4. Locate front left peg of tray into the hole at the end of the drive arm.



5. Slide the rear left peg into the horizontal slot on the left-hand side of bracket at the back of oven. Assembly is now complete and ready to operate.



CHAPTER 4
HYBAID SHAKE 'N' STACK
Methodology: Hybridisation Using the Hybaid Shake 'n' Stack

Placing Membranes in a Bottle

1. Place the Thermo bottles to be used into the Oven during the warm up period (approximately 1 hour).

2. Select a piece of support mesh appropriate for the size of the membrane. The recommended hybridisation mesh is supplied by Thermo *(see consumable listing in Chapter 8).*

3. Pre-wet the mesh and hybridisation membrane in a suitable tray containing 2 x SSPE (SSC) (see *Figure 4.1*).

4. Ensuring that the hybridisation membrane exactly overlays the mesh, roll both up into a tight roll.

   If more than one membrane is to be hybridised in a bottle, simply overlay further meshes and membranes as required before rolling. It is important that each membrane is separated from any other by a piece of mesh. Up to five 20 x 20mm membranes can be hybridised in a single hybridisation bottle.

5. Place 10-15mls SSPE (SSC) into a hybridisation bottle and then insert the roll in such a way that the leading edge (inside the roll) and the trailing edge are positioned relative to each other as shown in Figure 4.3).

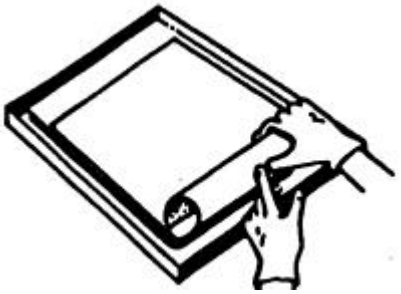
6. Place the bottle on a flat surface and then slowly unwind the membrane and mesh around the inside of the bottle by rocking and gently rolling the bottle along the surface. No air bubbles should be visible between the membrane and the bottle. If bubbles are present, the membrane should be removed and re-rolled.

   The procedure should then be repeated more gently. Rock the bottle backwards and forwards to attach the first part of the membrane to the bottle. Then roll the bottle in order to unwind the mesh and membrane.

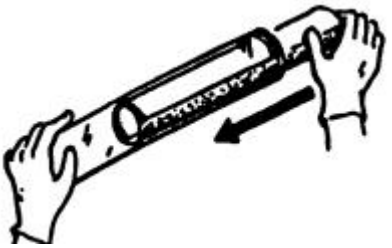
7. Continue until the membrane and mesh are fully unwound. The mesh ensures the probe will have access to all parts of the hybridisation membrane including those parts of the membrane, which overlap.

Figure 4.1: *Inserting membranes in bottle*

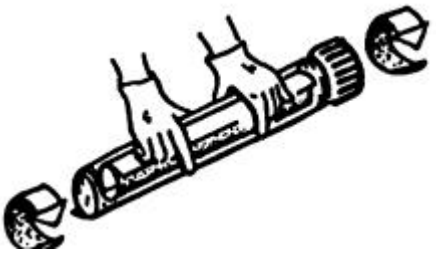
1. Shallow tray containing 2 x SSPE (SSC).
   Mesh.
   Membrane.
   Roll up mesh & membrane.



2. Place 10-15mls of SSPE (SSC) into a bottle and then insert the roll centrally.

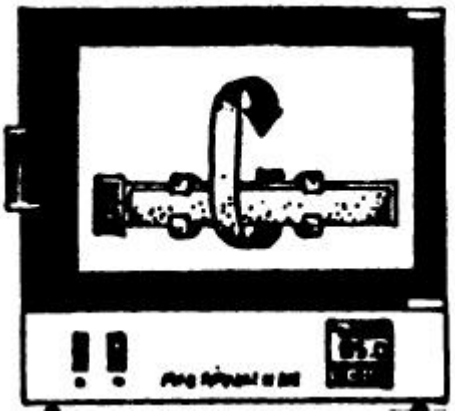


3. Secure cap and, holding bottle horizontally, roll to catch the trailing edge of the mesh; continue rolling in the same direction until coil of mesh and membrane is well positioned.



4. Pour out SSPE (SSC) and replace with pre-warmed pre-hybridisation buffer.

5. Place the bottle in the Oven so that it rotates in the Oven in the same direction, as it was unrolled in step 3.



Pre-Hybridisation

1. Once the membrane is in place in the bottle the SSPE (SSC) can be discarded and replaced with pre-hybridisation fluid.

This is easily done by removing the cap, pouring off the SSPE (SSC) and then pouring in the pre-hybridisation fluid. All the solutions used during hybridisation should be pre-warmed before use. 10-20ml solution is recommended for medium bottles and 5-10ml for small bottles. Higher volumes will be required if there is more than one membrane in the bottle, e.g. 15-25ml.

2. Replace the cap on the bottle and insert into the rotisserie.

Hybridisation

1. Denature the purified probe by boiling for five minutes, then store on ice. If the volume to be used for hybridisation is substantially different to that used previously for hybridisation in bags or boxes, ensure that the quantity of the probe is adjusted accordingly to maintain the correct probe concentration. If this is not done, high background may result.

2. Remove the bottle from the Hybridisation Oven and unscrew the cap.

3. If the same buffer is to be used for hybridisation as for pre-hybridisation simply pipette the probe into the pre-hybridisation fluid in the bottle. Take care to avoid pipetting the probe directly on to the membrane as this will result in hot spots. Alternatively, dilute the probe in pre-warmed hybridisation solution outside the bottle. Pour off the pre-hybridisation solution and replace with the probe solution.

4. Replace the cap and gently agitate the bottle to ensure an even distribution of the probe in the hybridisation solution.

5. Place the bottle back in the Hybridisation Oven, switch on the rotisserie and leave it to hybridise for the required time period.

Washing

Method 1 - Washing in Hybridisation Bottles

1. Remove the bottle from the Oven.

2. Pour off the hybridisation fluid and then **half fill** the bottle with the first pre-warmed wash solution (approx. 100ml) and replace the cap.

   NOTE: Room temperature washing or washing with solutions which have not been pre-warmed may result in background problems (see Appendix I of Thermo's Hybridisation Guide booklet).

3. Replace the bottle in the Oven and leave for the required time period.

   If transferring from sandwich box or plastic bag methodology, the times and temperatures are usually those which are currently being used, approximately 20 minutes per wash step.

4. Repeat steps 1-3 for each additional wash. The wash solutions, temperatures, etc., should be those recommended by the membrane manufacturer, or refer to the Thermo Hybridisation Guide.

   NOTE: All wash solutions should be pre-warmed for best results.

Method 2 - Washing in a Sandwich Box

Some scientists prefer to remove the membranes from the bottles and wash them all in one container. Washing may be performed very simply in the Hybaid Shake 'n' Stack on the shaking platform and is particularly useful when several membranes are being processed simultaneously.

1. Remove the hybridisation bottle from the Oven. Pour off the hybridisation fluid.

2. Using a pair of forceps, carefully remove the membranes and place them in a suitable container with a lid (e.g. sandwich box or similar).

3. Using the forceps gently unroll the membranes.

4. Add a sufficient volume of the pre-warmed first wash solution to totally immerse the membranes. Replace the lid and shake on the shaking platform in the Hybaid Shake 'n' Stack Oven at the required temperature for the specified time period.

   NOTE: Initial room temperature washes are not recommended and may result in subsequent background problems.

5. Remove the first wash solution and replace it with an equal volume of the pre-warmed second wash solution. Replace the lid and shake at the required temperature for the required time period.

6. Step 5 should be repeated for any subsequent wash. The washing protocol using the shaking platform will be identical to that used in the bottles.

Please refer to Appendix I for additional information on Washing.

In addition to bulk processing of blots during stringency washing steps, the shaking platform is ideal for performing several other stages of the blot generation and processing procedures. Three examples are given as follows: -

1. Pre-treatment of Gels prior to Nucleic Acid Transfer

   Gentle agitation of the gel during pre-treatment steps is essential to prevent damage to the gel. Pre-treatment steps are usually performed at ambient temperature.

   (i) Depurination - 0.25M HCl 10 minutes

   (ii) Denaturation - 1.5M NaCl, 0.5M NaOH, 30 minutes

   (iii) Neutralisation - 1.5M NaCl, 0.5M Tris Cl pH 7.2, 30 minutes

2. Pre-washing of Filters prior to Colony Blot Hybridisation

   Pre-washing is often advantageous to remove colony debris and hence reduce background noise when screening bacterial colonies by hybridisation. After lysing the bacterial colonies and fixing the DNA on the membrane, pre-wash in a solution of 2 x SSC/0.1% SDS at 65°C. Use several changes of buffer.

   After several washes residual colony debris may be removed by gently rubbing filter with a gloved finger.

3. Probe Stripping from Filters after Hybridisation

   An example of probe stripping procedure is as follows: -

   Wash the filter at 65°C in 5mM Tris Cl pH8.0, 0.2mM EDTA,
   0.1 x Denhardt's reagent for 1-2 hours.
   Check for residual activity by autoradiography, then re-probe.

CHAPTER 5
HYBAID SHAKE 'N' STACK
Rotisseries

Variable Axis Rotisserie

The Hybaid Shake 'n' Stack is equipped with Thermo's variable axis rotisserie. This allows the user to alter the angle at which bottles can be held in the rotisserie. A small offset from horizontal of between 1-15° causes fluid to flow from end to end within the bottles during rotation (*see Figure 5.1*). This "wave" motion provides a more vigorous and active hybridisation or washing stage and can allow probe volumes to be reduced to as low as 2-5mls. To achieve the optimum active wave conditions requires adjustment of the rotisserie angle. Note that a large angle offset from horizontal (15°) combined with a low probe volume (<1-2ml) can produce dry areas on the membrane and so should be avoided.

To alter the bottle angle: -

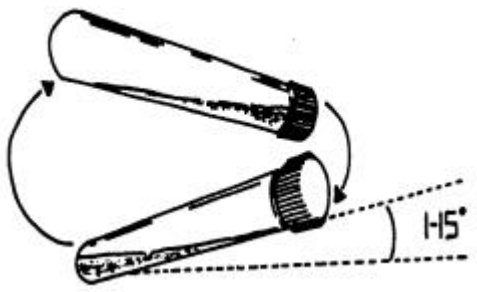
1. Release the two securing screws visible on the front rotisserie wheel using a cross-head screwdriver.

2. Put the rotisserie into the Oven and place a bottle in the rotisserie.

3. Rotate the free wheel to the desired angle.

4. Remove rotisserie and tighten the screw in the new position.

Figure 5.1: *Variable Axis Rotation*



50ml & 15ml Tube Rotisseries

Rotisseries are available that can hold 15ml and/or 50ml disposable tubes. *Details are given in Chapter 8.*

Tubes should be inserted into the rotisseries by sliding the tube sideways into the rotisserie. Care should be taken not to push the tubes too firmly into the rotisseries, otherwise the tube will crack.

CHAPTER 6
HYBAID SHAKE 'N' STACK
Cleaning & Decontamination

All items, which come into contact with a labelled probe, should be decontaminated before re-use. In many cases, a short rinse in water will be sufficient. In some instances, however, further decontamination procedures will be required.

Meshes

The quantity of radioactivity adhering to the mesh can be minimised by leaving the mesh in with the membrane throughout the washing procedure. Any radioactivity remaining can then be stripped from the mesh by the following procedure: -

1. Strip wash the mesh by incubating it in distilled water at 65°C in a shaking water bath for 15 minutes. Repeat.

2. If the mesh is still contaminated, soak it in a dilute solution, such as Decon 90, overnight.

3. Remove the detergent and proceed with two washes in distilled water for 10 minutes each.

4. If the mesh has been decontaminated no further action is necessary. If, however, the mesh is still contaminated, it will be necessary to leave it to decontaminate in Decon 90 for a longer time period.

5. Meshes should be allowed to dry flat between uses.

Bottles & Caps

The hybridisation bottles and caps can be decontaminated by the following procedure: -

1. Soak the bottles and caps in a dilute detergent solution (Decon 90) overnight.

2. Remove the Decon 90 and rinse with distilled water.

3. If the bottles and caps have been decontaminated, no further action is necessary. If, however, they are still contaminated, gently scrub them with an abrasive cloth or

brush and if necessary, leave to decontaminate in Decon 90 for a longer time period.

Oven Interior/Shaking Platform

Stainless steel drip trays are provided with all Thermo Ovens and are intended to contain spillages in the event of an accident. These, together with the stainless steel surfaces of the Ovens and the shaking platform, can be decontaminated by wiping clean with Decon 90, followed by distilled water. No further action should be necessary.

Rotisserie

The rotisserie may be cleaned with Decon 90 and distilled water. No further action should be necessary. Avoid alcohol or other organic solutions, which may affect the plastic surfaces.

Before using any cleaning or decontamination method except those recommended by the manufacturer, users should check with the manufacturer that the proposed method will not damage the equipment.

CHAPTER 7
HYBAID SHAKE 'N' STACK
Use of Non-Radioactive Probes

Thermo's Hybridisation Ovens have been tested with a variety of non-radioactive probes, e.g. the DIG Non-Radioactive Nucleic Acid labelling and detection system from Boehringer Mannheim.

Please refer to the Hybridisation Guide for guidelines on the use of non-radioactive systems.

NOTE: Refer to notes on the use of hazardous chemicals in the Warranty Section.

CHAPTER 8
HYBAID SHAKE 'N' STACK
Technical Specifications & Ordering Information

Temperature

Range	Ambient +8°C to 85°C
Selection	Digital
Display	Digital
Monitor/Sensor	Thermistor
Uniformity (in bottle)	±0.25°C
Accuracy (at 55°C)	±1.0°C
Safety Over Temp Cut Out	Yes
Alarm	Yes, audible (5°C above set temp)

Rotisserie

Rotisserie Speed	5-15 rpm
Capacity	10 large, medium & small bottles
Material	Stainless steel shaft
	Delrin plastic rotisserie wheels
	Variable axis 0-15°

Shaker

Speed	4-10 spm
Maximum Weight Capacity	1kg
Maximum Load Dimensions	250mmW x 180mmH x 200mmD
Material	Stainless steel

Dimensions

External	435mmW x 425mmH x 380mmD
Internal	345mmW x 245mmH x 240mmD
Weight	17.6kg (220V)
	21.1kg (110V)

Features

Plastic Snap-fit Rotisserie
Easy Set Temperature Controller

Standard Accessories

Shaking Tray Drive Arm Assembly
Shaking Tray Bracket
Power Cable
35mm Rotisserie
Drip Tray
1 Medium Sized Bottle (250mm x 35mm)
1 Bottle Gripper

Ordering Information

DESCRIPTION	CATALOGUE NO.
HYBAID SHAKE 'N' STACK **Includes:** Drip tray Delrin plastic rotisserie	HBSNSR 110/220
HYBAID SHAKE 'N' STACK **Includes:** Shaking platform Drip tray Delrin plastic rotisserie	HBSNSRS110/220
ACCESSORY PACK 2 medium bottles Bottle Gripper Mesh Hybridisation Guide	HBSNSAP1
ADDITIONAL ACCESSORIES **ROTISSERIES**	
35mm rotisserie to fit the Hybaid Shake 'n' Stack, Mini and Mini 10 Hybridisation Ovens. Holds up to 10 x large, medium & small bottles	HBMOVR35
Rotisserie to fit the Hybaid Shake 'n' Stack, Mini and Mini 10 Hybridisation Ovens. Holds up to 4 extra large bottles (70mm diameter)	HBMOVR70
Rotissieries to fit the Hybaid Shake 'n' Stack, Mini, Mini 10, Midi Dual 14 & Maxi Hybridisation Ovens. Holds up to 25 x 15ml tubes & 30 x 50ml tubes	HBOVR1
Rotisserie to fit the Hybaid Shake 'n' Stack, Mini, Mini 10, Midi Dual 14 & Maxi Hybridisation Ovens. Holds up to 50 x 15ml tubes & 16 x 50ml tubes	HBOVR2
Rotisserie to fit the Hybaid Shake 'n' Stack, Mini, Mini 10, Midi Dual 14 & Maxi Hybridisation Ovens. Holds up to 44 x 50ml tubes	HBOVR3
Shaking Platform	HBMOVP1

DESCRIPTION	CATALOGUE NO.
HYBRIDISATION BOTTLES	
Extra large bottle, 70 x 300mm	HBOVBXL
Large bottle, 35 x 300mm	HBOVBL
Medium bottle, 35 x 250mm	HBOVBM
Small bottle, 35 x 150mm	HBOVBS
Bottle gripper: 300mm length for extra large bottles	HBOVBGXL
Bottle gripper: 300mm length for large & medium bottles	HBOVBGL
Bottle gripper: 150mm length for small bottles	HBOVBGS
NYLON MESH	
Large mesh sheets, 23 x 23cm (qty 5)	HBOVLM5
Large mesh sheets, 23 x 23cm (qty 20)	HBOVLM20
Small mesh sheets, 10 x 15cm (qty 5)	HBOVSM5
Small mesh sheets, 10 x 15cm (qty 20)	HBOVSM20
Roll of mesh, 5m x 25cm	HBOVRM

APPENDIX I
HYBAID SHAKE 'N' STACK
Troubleshooting Guide for Nucleic Acid Hybridisations using Thermo Ovens

The Thermo bottle system is intrinsically simpler and safer to use than other methods of hybridisation such as hybridisation in bags or plastic boxes. In Thermo Hybridisation Ovens, the temperature of the solutions is precisely controlled and regulated, and, the rotisserie device ensures that the solutions are continuously and evenly distributed over the membrane. Thus, the optimum conditions for hybridisation and washing are maintained throughout when using this system. However, during the transfer of protocols from bags to bottles some minor adjustments to the protocols may be necessary.

When loading the mesh and membranes into the bottles, air bubbles should be avoided. Ensure that the Oven is sited on a flat surface so that the probe solution is distributed evenly along the length of the bottles and that there is sufficient probe solution to cover the entire membrane. On occasions the mesh and membrane can become tightly rolled up in the bottle. This occurs if the mesh is loaded incorrectly *(see Figure 4.1 in Chapter 4).*

Background Reduction - General

All solutions for nucleic acid hybridisations should be prepared using distilled water and highest quality reagents in clean glassware. In particular, water with a high organic content will cause bad background problems. Formamide should be freshly de-ionised. Membranes should always be handled wearing gloves or with forceps. The following considerations should also be applied: -

Pre-Hybridisation Procedure

Pre-hybridisation is required to block the sites on the nylon membrane, which the probe would otherwise bind to non-specifically. Failure to carry out adequate pre-hybridisation results in high backgrounds. If dextran sulphate is used in the hybridisation solution, then it must also be included in the pre-hybridisation solution.

Washing Procedure

Stringency washing should be carried out using large volumes (approximately 100ml) of the following solutions, which should be pre-warmed to the required temperature: -

1. 2 x 15 minutes with 2 x SSPE (SSC), 0.1% SDS at 65°C

2. 1 x 30 minutes with 2 x SSPE (SSC), 0.1% SDS at 65°C

3. 1 x 10 minutes with 0.1 x SSPE (SSC), 0.1% SDS at 65°C

All wash solutions should be pre-warmed to the appropriate temperature. An initial room temperature wash is not recommended and can cause background problems.

The final wash is a high stringency wash. Use of a hand held monitor to give an indication of the decrease in radioactivity as the washes progress is recommended and should give some indication as to whether this final wash should be carried out.

In general terms, the stringency of hybridisation and washing steps is increased by increasing the temperature, or by decreasing the salt concentration. Hybridisation should be carried out under relatively low stringency conditions compared to the washing procedures. It is generally simpler and more effective to adjust the stringency during the washing steps by altering the salt concentration rather than the temperature.

Probe Preparation

The final probe concentration should be in the region of 25-50ng/ml of hybridisation solution, at approximately 1-5 x 10^6cpm/ml.

The optimum length of probe is approximately 500-800bp. Purification of the labelled probe to remove unreacted triphosphates will reduce background problems and is recommended for all hybridisations. Probe solutions should be pre-warmed to the hybridisation temperature and care should be taken to ensure the membrane is not exposed to the concentrated probe solutions if adding it directly to the bottles.

Summary of Factors Resulting in High Backgrounds

1. Hybridisation solutions and/or wash solutions not pre-warmed before use.

2. Probe concentrations too high or probe not denatured. When transferring hybridisation protocols to bottles the volumes will be reduced. Ensure that probe concentrations are adjusted accordingly.

3. Unincorporated nucleotides not removed from probe solution.

4. Insufficient pre-hybridisation or blocking agents in pre-hybridisation and hybridisation solutions (e.g. Denhardt's reagent and salmon sperm DNA). An adequate pre-hybridisation is important to block non-specific hybridisation to the membrane.

5. Hybridisation and/or washing conditions not stringent enough: -

   (i) Decrease salt concentration.

   (ii) Increase temperature.

   (iii) Increase concentration of SDS.

   (iv) Increase wash times.

6. Membranes drying out. This may often be the cause of an apparent overlap problem and may result from: -

   (i) Too low a probe volume.

   (ii) Too slow a change over of solutions, particularly when bulk processing.

   (iii) Oven not level.

   (iv) Excessive variable axis angle.

7. Residual agarose on membranes may cause foggy backgrounds. Membranes should be rinsed in 2 x SSC to remove residual agarose and excess salt after blotting and prior to fixing (especially following vacuum blotting).

8. Multiple filters not separated by mesh in bottles.

9. Autoradiography problems. Random black spots and "lightening flash" markings on autoradiographs may be due to static electricity.

Summary of Factors Resulting in a Signal Lower than Expected

1. Insufficient exposure time of membrane to film during autoradiography.

2. Inefficient transfer and/or binding of nucleic acids to the nylon membrane.

3. Target sequence present at very low copy numbers. Increase the amount of sample loaded on to the gel.

4. Probe sequence not present in sufficient quantities. Increase the concentration of probe or include 10% dextran sulphate, which reduces the solvent volume and has the same effect.

5. No probe homology.

6. Double stranded DNA probe was not denatured - see standard protocols. Alternatively, probe degraded. This is more likely to occur when using RNA probes.

7. The specific activity of the probe was too low. Consider factors such as the probe concentration during the labelling reaction, half-life of radiolabelled triphosphates, etc.

8. Hybridisation and/or washing conditions were too stringent: -

   (i) Increase salt concentration.

   (ii) Decrease temperature.

   (iii) Reduce concentration of SDS.

   (iv) Reduce wash times.

9. The hybridisation time was too short.

APPENDIX II
HYBAID SHAKE 'N' STACK
Hybridisation Bottle Safety

Use of the Bottle Gripper

(Catalogue No HBOVBGS - to fit 15cm, small bottles)
(Catalogue No HBOVBGL - to fit 30cm, medium & large bottles)
(Catalogue No HBOVBGXL - to fit 30cm, extra large bottles)

This silicone rubber safety sleeve fits neatly over all our standard bottles and is included with every Thermo Oven we ship.

- Helping you to safely tighten and unscrew caps.
- Making handling of hot bottles easier.

For more details and ordering information, contact your nearest sales office:
UK and Europe: +44 (0) 1256 817282
US and Rest of the world: +1 866 984 3766
mol.biol@thermo.com

Bottle Care

Thermo Hybridisation Bottles are made of thick walled borosilicate glass, which protects users from radiation and has excellent long-term reliability.

☑ It is important to check your bottles regularly for chips, stress fractures and cracks. If these occur, the bottle must be discarded.

☑ Ensure bottles are stored either in a suitable rack or with caps replaced in between experiments. This will protect the bottle and sealing area.

☑ Replace "O" rings when worn or leaky. Replace all "O" rings every six months.

☑ Wear protective gloves to protect your hands in the event of accidental breakage.

☒ Never over tighten caps on bottles. Hand tight, is sufficient.

☒ If the bottle cap is difficult to unscrew **NEVER ATTEMPT** to force the bottle cap open. Allow the bottle to cool and retry. If the cap remains stuck, discard the bottle.

☒ The bottles should not be used at temperatures above 70°C.