HB-500 Minidizer
Hybridization Oven

Instruction Guide

An Analytik Jena Company

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81-0169-02 Rev H

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Introduction

The HB-500 Minidizer Hybridization Oven is a cost-effective, personal desktop hybridization oven with a capacity of four 35x150mm bottles, eight 50ml or 15ml conical plastic tubes. The Minidizer operates with a state-of-the-art microprocessor as well as temperature and variable speed controls. The HB-500 Minidizer enables consistent saturation of samples, whether it for washing or hybridizing. The bottle carousel rotates at a uniform speed of 12 RPM.

A touch-sensitive keypad and microprocessor provide accurate temperature control from ambient +10°C to 78°C. The keypad is located below a large LCD readout. Once the desired hybridization temperature is entered, the readout displays the current temperature inside the chamber. The chamber environment is calibrated at 78°C; at this temperature, the microprocessor accuracy is ±0.5°C. The uniformity of the chamber at 68°C is ±0.1°C; at 55°C is ±0.1°C; and at 42°C is ±0.1°C.

WARNING!

There may be build up of pressure within the hybridization bottles when they are taken from ambient to hybridization temperature. To help in relieving some of this pressure, preheat the solutions and bottles. Also, ensure that bottles are opened at the same temperature as the hybridization process. Do not allow the bottles to cool before opening.

To assure hybridization bottles remain leak proof and pressure proof, prevent temperatures above 70°C. If temperatures above this are used without first relieving the pressure within the bottle, there is a risk that bottles will leak and/or break due to internal pressure build up.

If the bottles are accidentally taken above 70°C without relieving the pressure, DO NOT open the hybridizer door. Turn off the hybridizer and allow it to cool before opening the door and checking the contents of the oven. Should the bottles be used above 70°C, relieve the pressure by unscrewing and re-tightening the cap.

If using radioactive material see the “Decontamination” section of this manual under “Service Procedures”.

Hybridization Oven Specifications

HB-500 Minidizer Hybridization Oven

Part Number	Volts/Hz
95-0330-01	115V/60Hz
95-0330-02	230V/50Hz
95-0330-03	100V/50Hz

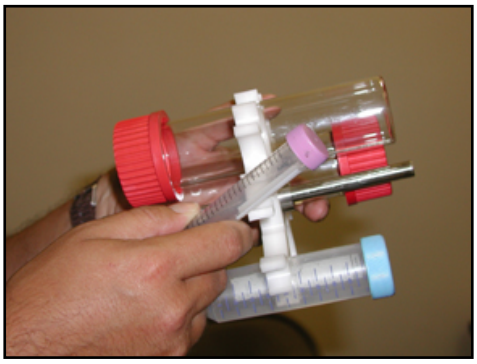
Specifications

Net Weight:	11.3 lbs (5 kg)
Temperature:	Ambient +10°C to 80°C
Heating Element:	500 watts
Temperature Display:	LCD
Rotation Speed:	12 RPM
Bottle Capacity:	Four (4) 35x150mm, Eight (8) 50ml, or Eight (8) 15ml
Dimensions:	9”W x 13”H x 8”D (229 x 330 x 203mm)

Installation

The Minidizer Hybridization Oven has capacity for four 35x150mm bottles and eight 50ml or 15ml conical plastic tubes. Combinations of multiple-sized bottles may be used at the same time.

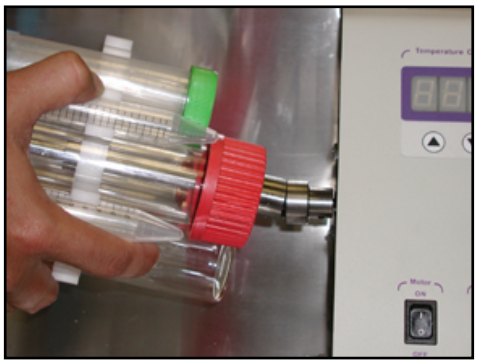
1. To insert bottles or tubes into the carousel, gently press them into the clips.



Installing the bottles and tubes

2. To insert bottle holder, begin by placing the large end of the bottle holder into the slotted holder on the right wall. Twist until the shaft locks into place, then slide the rod end into the black holder on the left wall. Compress the spring-loaded locking pins and rotate until aligned with the spindle on the right wall.

NOTE: ALWAYS USE AN EVEN NUMBER OF BOTTLES AND LOAD BOTTLES OPPOSITE EACH OTHER TO BALANCE THE BOTTLE HOLDER. THIS PREVENTS EXCESSIVE WEAR OF THE BEARINGS AND DRIVE MOTOR.



Installing the carousel

Operation

Caution: This equipment is not intended for interconnection with any other devices. Use of this equipment other than as intended may create a safety hazard and/or malfunction.

Using the Hybridization Oven

1. Place the unit on a level working surface and provide adequate room in front of the door to allow easy opening.
2. Plug the female end of the power cord into the back of the unit and the male end into a surge-protected outlet.
3. To turn on the main power supply, press the Power switch toward “ON” on the right side of the control panel.
4. To turn on the motor control to rotate the carousel, press the motor control power switch to the “ON” position.

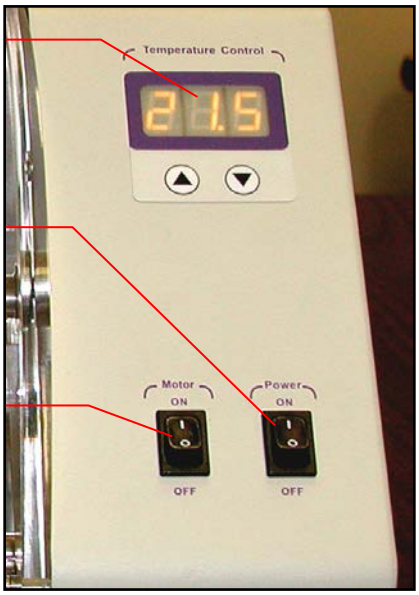
Temperature Calibration Controls

When the power is turned on, the software revision number flashes for several seconds. The display will then show internal surface temperature (not air temperature) for a few seconds. The display will then revert to the factory preset temperature of 30°C.

Temperature Display: Press the up and down arrows to set the temperature

Power: Press the power switch to the “I” position operate the temperature

Motor: Press the power switch to the “I” position rotate the carousel



Altering the Temperature Setpoint

The current setpoint value can be altered using the “UP” and “DOWN” buttons while the setpoint is being displayed. To change the setpoint from normal mode, proceed as follows:

1. Press then release either the “UP” or the “DOWN” button. The LCD will flash to indicate that the displayed value is the current setpoint. If either button is held down for more than several seconds, setpoint value will increase or decrease incrementally.
2. When the desired setpoint is reached, wait approximately five seconds without pressing either button; the display will revert to normal mode showing the actual temperature. The new setpoint becomes effective. When the unit is turned off and turned on again, the last used setpoint will be displayed.
3. Allow sufficient time for warm-up prior to hybridization procedures.

Temperature Calibration

The hybridization oven is calibrated at the UVP factory. UVP recommends temperature recalibration be performed at the UVP factory as recalibration by the user may void the warranty. To return the unit to UVP, first obtain a RGA (Returned Goods Authorization) number from UVP.

Call UVP’s customer service department in Upland, California at (800) 452-6788 or (909) 946-3197 or Cambridge, UK at +44(0)1223-420022 to obtain an RGA number or to inquire about temperature calibration.

Service Procedures

Care and Cleaning

NOTE: ALWAYS UNPLUG THE UNIT FROM THE ELECTRICAL SUPPLY BEFORE CLEANING OR DRYING THE UNIT!

The units are built to provide trouble-free operation for the life of the system. To ensure correct operation:

- Wipe any water from inside and outside the unit with a soft cloth or sponge.
- Use soap and water with a soft cloth or sponge to clean the unit.
- Do not allow chemicals to remain on unit surfaces.
- Never clean unit with abrasive pads or cleaners.
- Never clean unit with acetone or chloroform.

Bottle Care

UVP's hybridization bottles are made of thick walled borosilicate glass which helps protect users from radiation and has excellent long-term reliability.

- It is important to check the bottles regularly for chips, stress fractures and cracks. If this occurs, the bottle must be replaced.
- 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/PTFE seals when worn or leaky; replace O-rings or PTFE seals every six months.
- Wear protective gloves to protect your hands in the event of an accidental breakage.
- Never over tighten caps on the bottles. Hand tightening is sufficient.
- If the 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.

Decontamination

Bottles and Caps*

- Soak items in a diluted detergent solution overnight.
- Remove from detergent and rinse items with distilled water.
- If items are still contaminated, gently scrub with an abrasive cloth or brush. If necessary, continue to soak items in the detergent solution for a longer period of time.

Oven Chamber*

The oven chamber and drip tray may be decontaminated by wiping clean with a decontaminating agent, then repeating with distilled water.

***NOTE**: These decontamination methods may not remove all contaminants. Refer to Federal, State and local guidelines and biological protocols to ensure decontamination.

NOTE: If any unit requires service, a Returned Goods Authorization number (RGA) must be obtained from UVP's Customer Service department prior to returning any item to UVP. If radioactive or biological hazardous materials have been present within the unit, radioactive decontamination and biological cleanup as per current Federal, State, and local guidelines and biological protocols must be performed BEFORE returning the unit.

Replacement Parts and Accessories

Replacement parts lists are provided below for the HB-500 Minidizer Hybridization Oven. Repairs or replacement other than specified in the following procedures shall be done only by authorized service personnel.

PART NUMBER	DESCRIPTION	QUANTITY
76-0070-02	Bottle Carousel Holder	1 Required
63-0024-02	Carousel Shaft Assembly	1 Required
68-0088-01	Carousel Pillow Block	1 Required
46-0024-02	Motor Drive Coupler Assembly	1 Required
56-0022-01	Fuse, 3AMP/250V 5x20mm (Sold Individually)	2 Required
88-0004-01	Bottle Cap and O-Ring/PTFE Seal	
07-0194-01	Borosilicate glass bottle with polypropylene cap and PTFE seal, 35 x 150 mm	
58-0085-01	Power Cord (115V)	
58-0085-03	Power Cord (230V)	

Technical Support

UVP offers expert technical support on all UVP products. If there are any questions about product use, operation or repair, contact UVP's offices at the locations below.

NOTE: A Returned Goods Authorization (RGA) number must be obtained from UVP's Customer Service prior to returning any product.

If you are in North America, South America, East Asia or Australia:	**If you are in Europe, Africa, the Middle East or Western Asia:**
Call (800) 452-6788 or (909) 946-3197, and ask for **Technical Support** during regular business days, between 7:00 am and 5:00 pm, PST.	**Call +44(0) 1223-420022**, and ask for **Customer Service** during regular business days between 9:00 am and 5:30 pm.
E-mail your message to: info@uvp.com or techsupport@uvp.com	**E-mail** your message to: uvp@uvp.co.uk
Fax Technical Support at (**909) 946-3597**	**Fax** Customer Service at **+44(0) 1223-420561**
Write to: UVP, LLC. 2066 W. 11th Street, Upland, CA 91786 USA	**Write to**: Ultra-Violet Products Ltd. Unit 1, Trinity Hall Farm Estate, Nuffield Road, Cambridge CB4 1TG UK

Warranty

UVP's quality hybridization ovens are guaranteed to be free of defects in materials, workmanship and manufacture for one (1) year from the date of purchase. Consumable and disposable parts including, but not limited to bottles, tubes and filters, are guaranteed to be free from defects in manufacture and materials for ninety (90) days from the date of purchase. If equipment failure or malfunction occurs during the warranty period, UVP shall examine the inoperative equipment and have the option of repairing or replacing any part(s) which, in the judgment of UVP, were originally defective or became so under conditions of normal usage and service.

No warranty shall apply to any instrument, or part thereof, that has been subject to accident, negligence, alteration, abuse or misuse by the end user. Moreover, UVP makes no warranties whatsoever with respect to parts not supplied by UVP or that have been installed, used and/or serviced other than in strict compliance with the instructions appearing in the operational manual supplied to the end user.

In no event shall UVP be responsible to the end user for any incidental or consequential damages, whether foreseeable or not, including but not limited to property damage, inability to use equipment, lost business, lost profits or inconvenience arising out of or connected with the use of instruments produced by UVP. Nor is UVP liable or responsible for any personal injuries occurring as a result of the use, installation and/or servicing of equipment. This warranty does not supersede any statutory rights that may be available in certain countries.

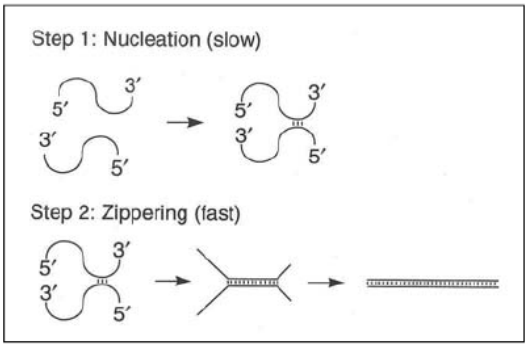
Hybridization Techniques

There are really two main steps to a hybridization reaction: hybridizing two strands of complementary DNA, and detection of the hybridized DNA. Nucleic acid hybridization is a mechanism where strands of DNA in a single stranded state have their complements bind together. The proximity of the DNA strands to each other determines the frequency of the binding events and, in fact, successful binding is proportional to their concentration. The concentration of the target (nucleic acid) is the independent variable in all hybridization reactions.

Since the target concentration is usually the unknown variable, an excess of labeled probes (what you use to find the target) will drive the reaction, thus decreasing the time for the probe to hybridize to a target. This is simply increasing the chances of a probe bumping into a target. However, with an enormous amount of probe around (in the solution or on the surface of a membrane), the background signal will also be enormous. The typical approach to correct for excess background (noise) on a membrane or slide hybridization is to wash it in a low salt buffer, as this favors the disassociation of unbound probe from the membrane/slide and non-complementary DNA. In solutions, a probe can be enzymatically degraded by using a single strand-specific nuclease.

Mechanisms of Nucleic Hybridization

Hybridization occurs with a process called ***nucleation*** whereby the two separate nucleic acid strands come into close proximity of each other. A duplex region is formed where a minimum of three bases of one strand complements to those on the second strand. If the remainder of the strands are complementary, the two strands will anneal or zipper together very quickly. The rate-limiting step in nucleic acid hybridization is the duplex formation, which again explains why probe-to-target concentrations are critical.



Experimental Protocols

There are many different protocols available on the web, in journals, and in text references and we reference several at the end of this text.

1. Concentration of Species

Target: How much target molecule depends on the species you expect to find. Cellular constituents may be expressed in large or small amounts; the trick is to start with enough target (~25 μg) and determine experimentally.

Probe: Plan to have more probe than expected target. To answer questions about adding too much probe, run an extinction experiment: serially increase the amount of target by a factor of two and use a fixed amount of probe. Hybridize for a short length of time and quantitate the amount of probe that has hybridized. As long as the signal increases and shows linearity there is excess probe (Fig 2). If the signal levels off and a loss of linearity noticed, then the probe is not in excess (Fig 2).

2. Length of Probe

The goal is to increase hybridization efficiency while minimizing background. In most cases probes range from 20 – 1000 bps.

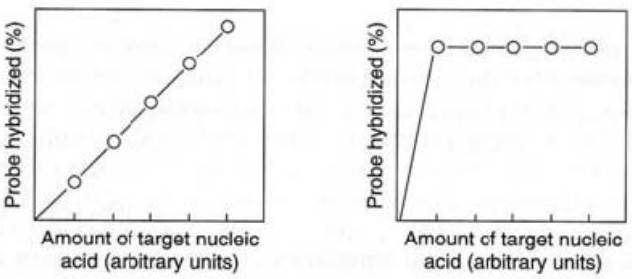


Figure 2. Analysis for probe excess in hybridization reactions.

3. Salt Concentration and Temperature

Nucleic acid requires salt (monovalent cations) to reduce the ionic effects of the phosphate backbone, and heat as a form of non-denaturing kinetic energy. Because the salt concentration and temperature effect each other, knowing the thermostability of the hybrid probe is helpful. Hybridization rate varies directly with the sodium ion concentration between 0.03 and 1.2 M. Most protocols run between 0.5 and
1.1 M Sodium.

Situation	Response
G+C = 45-55%	Follow normal protocol
G+C < 45%	Lower salt and temperature
G+C > 55%	Raise salt and temperature
Evidence of probe target mismatching	Lower salt and temperature
Target and probe is degraded on aqueous	Hybridize in a formamide-based buffer solution
Unacceptable high background	Use less probe Hybridize at lower salt/ higher temperature Wash with lower salt higher temperature Incubate with very low salt/change nuclease(solution) Use a smaller probe or a different probe Clean probe of contaminants prior to use

4. Aqueous or Denaturing Hybridization Buffer

If hybridization takes place in an aqueous salt environment of 0.8 to 1.2M salt, the T M½ (the temperature at which the half of the duplex molecules will dissociate under a given set of conditions) can be 90°C. This is high enough to degrade DNA, RNA and some proteins. It is therefore possible to add formamide as a denaturing / temperature lowering agent because for every percent of formamide in the reaction the TM½ is reduced by 0.65°C. Therefore, at 80% formamide, reactions can be performed in the 40 – 55°C range. However the rate of formamide-based hybridization is at least three-fold lower than that of aqueous hybridization requiring longer incubations.

Protocol 1:

Random Priming Method for Tagging DNA with Fluorescein-Labeled Nucleotide and Others

This method uses DNA polymerase to incorporate Fluorescene-11- dUTP into double stranded DNA probes. This protocol can be used to incorporate **any** tagged nucleotides.

Equipment

- Micropipettes and tips
- Boiling water bath
- 1.5 mL Microcentrifuge tubes
- Microcentrifuge
- Cap lock for Microcentrifuge tube
- Water bath set to 37°C

Reagents

- Deionized, sterile water
- EDTA, 0.5 M
- Klenow DNA polymerase , 4-5 units/µL
- Nucleotide mix (300µm each of dAT P, dCTP, dGTP and 60µm dTTP)
- Random nonamer (9-mer) primers, 2.5 µg/µL in water
- Reaction buffer, 10X: 50mM MgCl2, 10mM 2-Mercaptoethanol, 500 mM Tris-HCl, pH 7.5\
- Tagged nucleotide: fluorescene-11-dUTP
- Template DNA in water (5ng/ mL)

Procedure

1. Pipette 10 mL of template DNA plus 10 mL of water into a microcentrifuge tube and cap tightly. Cover cap with a cap lock or bend a paper clip in half and secure over the microcentrifuge tube.
2. Place the tube into the boiling water bath for 5 minutes.
3. Immediately place tube on ice for 5 minutes.
4. Centrifuge for 15 seconds in microcentrifuge.
5. Add the reagents listed below to a fresh tube on ice in the following order:

    a. 10 mL Nucleotide mix
    b. 5 mL Tagged nucleotide
    c. 5 mL Reaction buffer (x10)
    d. 5 mL Random primers
    e. 10 mL Boiled DNA
    f. 14 mL Water
    g. 1 mL DNA polymerase
    h. Mix gently and incubate at 37 °C for 1 hour
    i. Stop the reaction by adding 2 mL EDTA
    j. Store probes at -20 °C in the dark

Protocol 2:

Hybridization to Nylon or Nitrocellulose

Hybridization to nylon or nitrocellulose membranes containing Nucleic Acid is accomplished by adding single-stranded probe to the membranes that have been previously incubated with prehybridization solution. The prehybridization and hybridization solutions both contain buffers designed to prevent adventitious binding of the probe to the filters.

Reagents and Equipment

Prehybridization/hybridization solution [45% formamide, 5X SSPE (0.9 M NaCl, 50mM sodium phosphate buffer, pH 7.4, 5mM EDTA), 0.1% SDS, 5X Denhardt's solution (0.1% each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin), and 100 mg/mL of denatured salmon sperm DNA). Mix well and remove aggregates before use.

Notes: When preparing prehybridization/hybridization solutions, add dry reagents directly to the formamide/SSC solution. Incubate with mixing at 40-50°C for 2 hours or until dissolved. Store at 20°C. SDS will precipitate at room temperature but remain in solution at 37°C.

- UVP Hybridization bottle(s) and caps
- 15 mL plastic tube
- Boiling Water Bath
- Bucket of ice
- Gloves
- Plexiglas shield
- UVP Minidizer, HybriCycler, or Hybridizer Hybridization Oven

Procedure

1. Add 15 mL of prehybridization solution to each hybridization bottle containing the blot. Remove bubbles between the glass and blot. Cap the blots and close the Hybridizer.
2. Incubate the blot at 42 °C for 1 hour.
3. Remove prehybridization solution and replace with 10 mL of hybridization solution.
4. Pipette 1x106 counts per minute of radio labeled probe or 200ng of biotinylated DNA into a 15-mL plastic tube. Seal the tube with a plastic cap and poke a hole in the top with a syringe needle to prevent pressure build-up during boiling.
5. Denature the probe by placing the samples in the boiling water bath and heating for 10 minutes. Immediately transfer the tube to ice for 5 minutes (to prevent renaturation). Add 5 mL of hybridization buffer to the probe and transfer to the hybridization bottle containing the blot: AVOID pouring the probe directly onto the blot.
6. Incubate in the UVP HybriCycler, Hybridizer, or Minidizer 6 to 8 hours at 42 to 56 degrees.

Washing the Blot

- Tupperware container (sized to contain the blot)
- 0.1X SSC, 0.1% SDS (pre-warmed to 50 °C)
- 2X SSC, 0.1 % SDS (room temperature)
- 2X SSC (room temperature)
- 0.15X SSC, 0.1% SDS (pre-warmed to 50 °C)
- Gloves
- Filter Paper
- Cardboard
- Plastic wrap
- Tape

Non-Radioactive Probes

1. Wash blots in 2X SSC, 0.1% SDS for 3 minutes at room temperature (repeat one)
2. Wash filter in 0.15X SSC, 0.1%SDS for 15 minutes at 50 °C (repeat once)
3. Store blots in 2X SSC at room temperature

Radioactive Probes

Additionally you will need:

- X-Ray film holder
- X-Ray film
- Intensifying screen

Procedure

1. Remove blot from hybridization tube and transfer to Tupperware container
2. Rinse briefly in 50 °C 0.1X SSC, 0.1% SDS.
3. Remove this solution to radioactive waste and wash
4. Wash blot three more times in the same solution.
5. After the final wash, dry blot on filter paper for 10 minutes. This is a good time to quickly pass your hand held radioisotope reader (beta or gamma counter) over your blot to get a general idea as to the exposure time you will need for the X-ray film. Hot blots are 20 minutes to 2 hours. Not so hot blots can be left overnight (8 hours).
6. Tape the blot to a cardboard backing.
7. Cover with plastic wrap to prevent the blots from sticking to the X-ray film.
8. Place the cardboard containing the blots into the X-ray film folder.
9. In the darkroom, place a piece of X-ray film over the blot(s).
10. Place an intensifying screen on top of the film.
11. Close the film folder and clamp it.
12. Store at -70 °C. The low temperature reduces light scattering and increases the length of exposure time. Expose the blot for 20 minutes to 24 hours.

Protocol 3:

Chemiluminescence Detection: HRP-Tagged, Alkaline Phosphatase (AP) Probes or Antibody Conjugates

Equipment

- Clear plastic cling-wrap or Clear transparent sheet protector
- UVP EC3 or AC1 Darkroom with Cooled CCD camera
- Pipette

Reagents for Chemiluminescence

- ECL™ (or other) detection reagent 1
- ECL™ (or other if required) detection reagent 2
- Membrane following hybridization.

Procedure

1. Mix equal volumes of detection reagents 1 and 2
2. Pipette the mixture over the surface of the membrane and leave at room temperature for 1 minute.
3. Drain the sample and wrap it transfer to Darkroom
4. Close the darkroom and image using CCD camera.

References

Ross, J; Nucleic Acid Hybridization: Essential Techniques; 1998, John Wiley and Sons, ISBN 0-471-97125-1

Sambrook, J; Molecular cloning: a Laboratory manual; 1987, Cold Spring Harbor Laboratory Press; ISBN 0-87969-309-6

Current Protocols in Molecular Biology; 1987, John Wiley and Sons; ISBN 0-471-50338-X