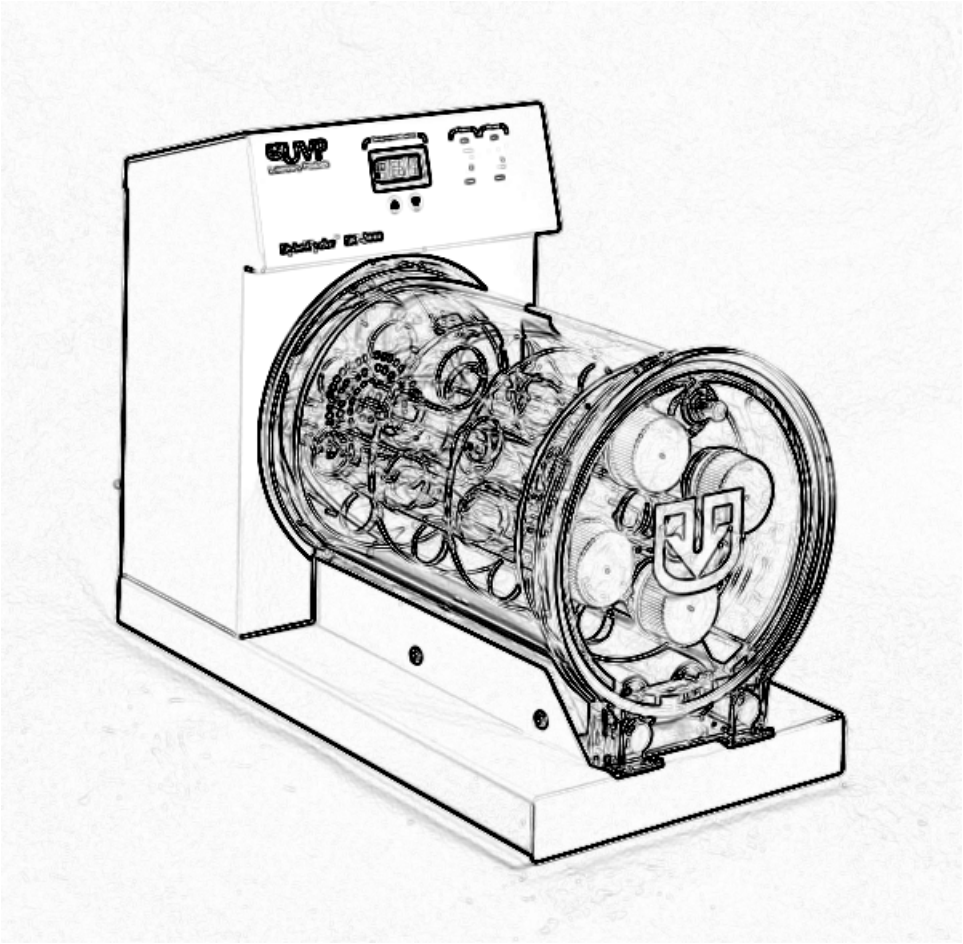
HybriCycler Hybridization Oven

Operating Instructions



IMPORTANT: Please read these instructions before operating your UVP HybriCycler™ system to familiarize yourself with operation procedures.

UVP

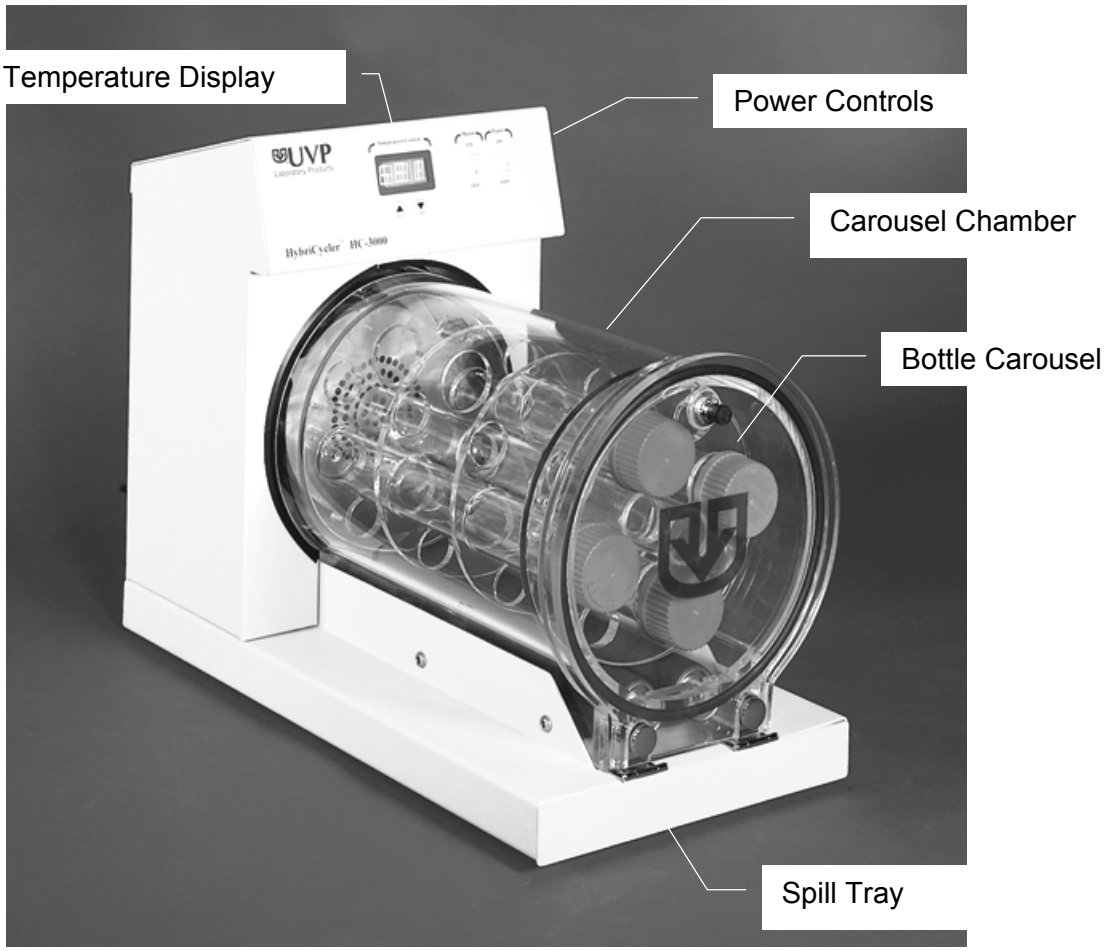
81-0187-01 Rev C

Introduction

The HybriCycler™ Hybridization Oven is designed as a compact alternative for hybridization requirements. The HybriCycler Oven features microprocessor operation for precise mixing capability. This space-saving design is manufactured with a 3/8” thick beta-blocking acrylic and polycarbonate cylinder providing 360° view of the bottles. The HybriCycler Oven housing is manufactured with a scratch-resistant, powder-painted epoxy finish for easy cleaning and maintenance.

The HybriCycler operates at 11 RPM, enabling consistent saturation of samples, whether is for washing or hybridizing. Having the ability to remove the carousel allows for additional bottle size capacity. An easily removable protective tray under the rocker wheel allows easy clean-up of spilled media.

A touch sensitive keypad and microprocessor control accurate temperature output from ambient +10°C to 80°C. The keypad is located just below a large LED readout. Once the desired hybridization temperature is entered, the readout displays the current temperature inside the chamber. The HybriCycler Oven features:



HybriCycler™ Hybridization Oven Hardware

The HybriCycler Oven is comprised of:

Hybridization Chamber

The space-saving Hybridization Chamber is manufactured of 3/8” thick beta-blocking acrylic providing a 360° view of the bottles.

Bottle Carousel

The cartridge-style bottle carousel is manufactured of ¼” thick polycarbonate. The carousel has the capacity to hold

- Four 35x300mm bottles
- Eight 35x150mm bottles
- Eight 50ml bottles

or one oversize bottle 70x300mm by removing the carousel.

Spill Tray

The drawer-style spill tray is designed to contain spills that leak from any of the bottles within the Hybridization Chamber. The spill tray has a fluid capacity of 300ml.

Set-Up Instructions

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

1. Plug the female end of the power cord to the connection on the back of the HybriCycler.

2. Insert your Hybridization bottle(s) into the bottle carousel. Start by sliding the bottle into the bottle carousel's entry hole at either end of the bottle carousel.

WARNING: Do not insert a bottle into the center tube. To do so would block air flow and may result in a safety hazard and or malfunction.

3. When the end of the hybridization bottle is about to meet the bottle carousel's exit hole on the opposite end, push the bottle down toward the center of the bottle carousel, which secures the bottle into the securing rings. Then slide the hybridization bottle through the bottle carousel's exit hole.

4. Open the HybriCycler's door by pressing down on the rear portion of spring-loaded door latch. Slide the bottle carousel into the hybridization chamber ensuring that the front disk is inserted into the groove of the carousel guide. Close the door by squeezing the door against the hybridization chamber until the spring-loaded door latch secures.

Operation

HybriCycler functions:

- The “Power” switch turns the HybriCycler ON or OFF.
- The “Motor” switch turns the bottle rotation ON or OFF.
- The “Temperature Control” displays and controls the temperature setting within the hybridization chamber.

1. To operate the unit, press the “Power” switch to the ON position.

2. Set the “Temperature Control” to the required temperature by pressing either the “Up” or “Down” keypad arrow buttons to raise or lower the displayed degree (displayed in degrees Celcius). This setting is adjustable from ambient +10°C to 80.0°C. See Temperature Control Instructions on page 6 for more details.

3. Press the “Motor” switch to the ON position.

Setting the Temperature Controls

Whenever power is applied to the controller, the software revision number is displayed for several seconds. While the software revision number is being displayed, the intensity of the display digits alternates between full and half brightness, and on some models the two outside decimals points blink on and off. After several seconds, the display reverts to showing the controlled temperature. In this mode, at most a single decimal point is illuminated, and the display intensity is steady.

Altering the 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:

- Momentarily press then release either the “UP” or the “DOWN” button. The LED intensity will alternate between half and full intensity to indicate that the displayed value is the current setpoint.

- Increase or decrease the setpoint value by pressing “UP” or “DOWN” buttons respectively. If either button is held down for more than several seconds, setpoint value will increase or decrease continuously.

- 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 and is stored in the non-volatile memory when the display reverts to the normal mode.

Calibrating the Temperature Reading

The HybriCycler is calibrated at the UVP factory. UVP recommends temperature recalibration be performed at the UVP factory during the warranty period as recalibration by the user may void the warranty. If the user chooses to recalibrate the unit, Temperature Calibration Reading instructions may be obtained from UVP by calling UVP’s customer service department in Upland, California at (800) 452-6788 or (909) 946-3197 or Cambridge, UK at +44(0)1223-420022.

Care and Cleaning

The drawer-style spill tray is designed to contain any spills that leak from any of the bottles within the Hybridization Chamber. The spill tray has a fluid capacity of 300ml. The inside of the HybriCycler Hybridization Chamber should be cleaned with a damp cloth. The bottle carousel and outside of the HybriCycler can be cleaned with soap and water. The chamber, rollers and spill tray may be decontaminated by wiping clean with a decontaminating agent*, then repeat with distilled water. Rollers and carousel guide are removable facilitate cleaning.

NOTE: *This decontamination method may not remove all contaminants. Refer to Federal, State and Local Guidelines and Biological Protocols to assure decontamination.

If any unit requires service, a **Returned Goods Authorization (RGA)** must be obtained from UVP's customer service department prior to returning any equipment to UVP. If radioactive or biological hazardous material has been used within the unit, radioactive decontamination and biological clean-up as per current Federal, State and Local Guidelines and Biological Protocols must be performed, BEFORE returning the unit to UVP.

Replacement Parts

Replacement parts lists are provided below for the HC-3000 HybriCycler Systems. Repairs or replacement other than specified in the following procedures shall be done only by authorized service personnel.

	Qty	Part Number
Fuse, 10 Amp, 250V 5x20mm, Slo-Blo	2 ea	56-0002-04
Main Motor Switch	1 ea	53-0165-02
Power Cord, 115V	1 ea	58-0085-01
Power Cord, 230V	1 ea	58-0085-03
Adjustable Feet	4 ea	72-0062-01
Carousel Assembly	1 ea	76-0089-01
Roller Assembly	2 ea	76-0309-01
Bottle, Small 35x150mm		07-0194-02
Bottle, Large 35x300mm		07-0194-01

Technical Support and Maintenance

UVP offers technical support for all of its products. If you have any questions about the product's use, operation, or repair, please call or fax UVP Customer Service at the following numbers:

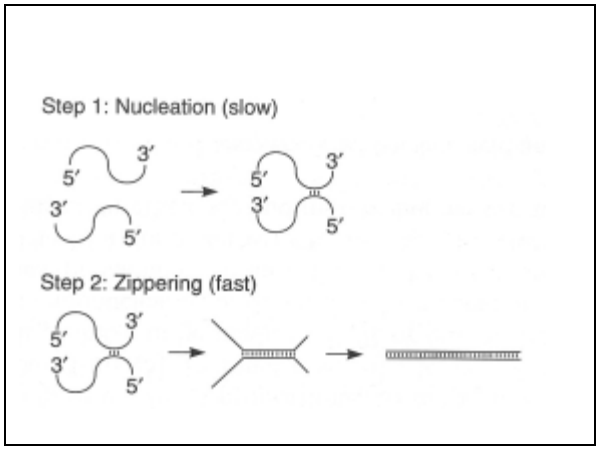
In the US toll free (800) 452-6788 or (909) 946-3197, fax: (909) 946-3597, email: info@uvp.com
In Europe/UK call +44(0)1223-420022 or fax: +44(0)1223-420561, email: uvp@uvp.co.uk

Hybridization Techniques

There are really two main steps to a hybridization reaction: hybridizing two strands of complimentary 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 is in fact successful binding is proportional to their concentration. The concentration of the target (nucleic acid you are looking for) is the independent variable in all hybridization reactions.
Since the target concentration is usually the unknown variable, an excess of labeled probe (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. But with an enormous amount of probe around (in 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: wash 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 left). If the signal levels off and a loss of linearity noticed, then the probe is not in excess (Fig 2 right).

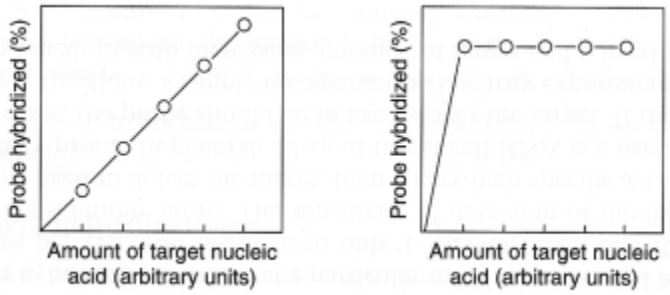


Figure 2. Analysis for probe excess in hybridization reactions.

2: Length of Probe

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

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 solution	Hybridize in a formamide-based buffer
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 $T_{M½}$ 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-lebeled 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 dATP, dCTP, dGTP and 60 μm dTTP)
- ❑ Random nonamer (9-mer) primers, 2.5 μg/μL in water
- ❑ Reaction buffer, 10X: 50mM $MgCl_2$, 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. Immeadiately 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:
6. 10 mL Nucleotide mix
7. 5 mL Tagged nucleotide
8. 5 mL Reaction buffer (x10)
9. 5 mL Random primers
10. 10 mL Boiled DNA
11. 14 mL Water

12. 1 mL DNA polymerase
13. Mix gently and incubate at 37°C for 1 hour
14. Stop the reaction by adding 2 mL EDTA
15. 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
- Glove
- Plexiglass shield
- UVP Minidizer™, HybriCycler™, or Hybridizer™

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 $1x10^6$ 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: UVP BioWest ™, HRP-tagged, Alkaline phosphatase (AP) probes or antibody conjugates

Equipment

- ❑ Clear plastic cling-wrap or Clear transparent sheet protector
- ❑ UVP EpiChemi II™ Darkroom
- ❑ Pipette
- ❑ Cooled CCD camera

Reagents

UVP BioWest™ – For HRP Tagged Species

- BioWest Substrate Working Solution: Prepare by mixing equal parts 1:1 BioWest Luminol/Enhancer Solution and BioWest Stable Peroxide Solution. This working solution is stable for a minimum of 24 hours at room temperature and may be stored in ambient light and temperature.

BioWest Procedure

1. Immerse the blot in BioWest Substrate Working Solution for five minutes. Completely immerse the blot in the substrate (0.125 mL/cm^2).
2. Remove the blot from the substrate solution and place it in a sheet protector.
3. Remove any bubbles between the blot and the surface of the protector.
4. Place in an EpiChemi II Darkroom and with all internal lights off image the blot.

Other Chemiluminescence

- ❑ ECL tm (or other) detection reagent 1
- ❑ ECL tm (or other if required) detection reagent 2

Product Warranty

UVP's quality HC-3000 HybriCycler System is 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 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.

NOTE: Slight visual aberrations in the acrylic are a natural occurrence during the manufacturing/forming process and are not considered a defect.

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