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Clean Spot PCR Workstation instruction manual

The CleanSpot Manual

INTENDED USE

The COY CleanSpot is a fully enclosed UV (254 nm) work area designed to irradiate reaction mixtures, reagents, tips, and small equipment often used in PCR* reactions.



THEORY OF OPERATION

The CleanSpot utilizes powerful overhead UV lights mounted in a unique reflector. The reflector focuses light emitted from the upper and lateral surfaces of the UV bulbs onto the bottom work area and unfolded shelf. Introduction of pyrimidine dimers and other photo damage into contaminating DNA sequences present in the work area and reaction mixtures will render them biologically inactive, thus creating a "clean" environment for the preparation of amplification reactions and helping "decontaminate" some of the reaction components.

UV irradiation is important when there is a potential for amplification of unwanted DNA sequences during cycles of PCR. UV light introduces pyrimidine dimers in adjacent pyrimidines along a strand of single-stranded or double-stranded DNA. The presence of dimerized pyrimidines prevents DNA polymerase from extending through these base pairs. AS a result the DNA is not extended to full length, inhibiting

geometric amplification of unwanted DNA targets. If wanted templates were accidentally added to the reaction mixture before irradiation, they would be rendered unamplifiable by current amplification procedures.

The duration of the UV dose, i.e., total energy required, will be dependent upon the concentration of contaminating sequences, the base pair length, and the sequence specificity of the DNA. Sarker and Sommer illustrated a logarithmic relationship between relative inactivation by UV light and the length of DNA targets. Targets that are longer and more water saturated are more susceptible to UV light and therefore require less irradiation to inactivate. The work area is large enough to irradiate several racks of tubes, pipettes, pipette tips, and other items required for setting up PCR reactions. Tubes should be uncapped and placed in a microtube rack in the center of the CleanSpot. A typical microtube contains 10X Reaction Buffer, Mg⁺⁺, nucleotides, and distilled water. Care should be taken not to put items in the shadow of the shelf. Exposure time may be shortened by putting items to be irradiated on the shelf.

The CleanSpot inactivates 10 ng of a 1.5 kb target DNA within 25-20 minutes. This time takes into account normal bulb degradation and non-uniformity. An average of 1020 mJ/cm² of UV light is delivered in 30 minutes to the bottom of the unit, in the areas not shaded by the shelf.

*PCR (Polymerase Chain Reaction) is covered by U.S. Patents issued to Hoffman La-Roche Corporation.

TECHNICAL SPECIFICATIONS

Dimensions: 18" x 24"W x 28"H
(46cm x 61 cm x 71 cm)

Weight: 31 pounds (14 kg)

Light Source: 2 UV bulbs – 254 nm
white fluorescent bulb

Electrical: Domestic: 110VAC,
128 Watts, 60Hz, 1.2A

International: 220VAC,
128 watts, 50HZ, 0.6A

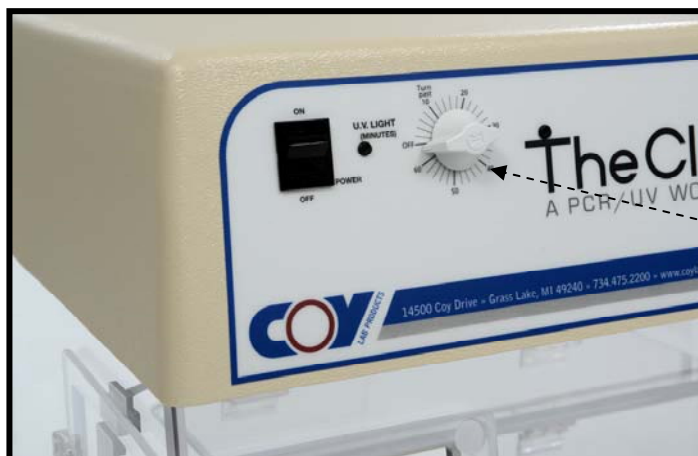
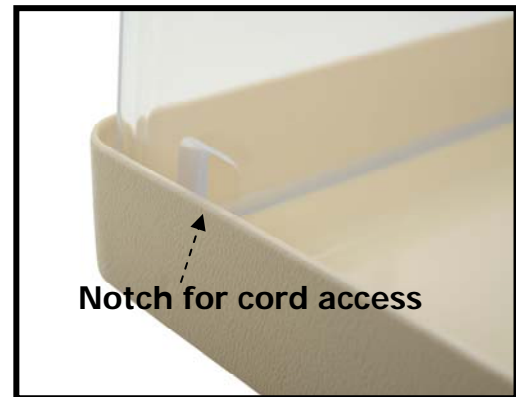
Fuses: Domestic: 1.5A
(slow blow)

International: 1.25A
(slow blow)

*Replace only with fuse of specified type
and current rating*

Warranty: 6 Months

Safety Device: Reed switch prevents
operation of UV lamps
when doors are open

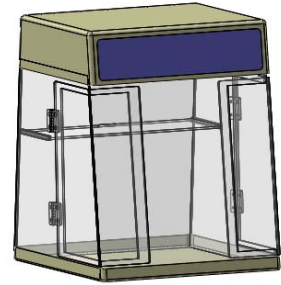


WARNINGS AND PRECAUTIONS

1. The CleanSpot uses two 254 nm UV light bulbs. Always wear protective clothing and safety goggles. DO NOT expose skin to UV light.
2. When using the shelf, the area under the shelf will not be exposed to UV rays, therefore do not place items under the shelf. The shelf is removable.
3. Some micropipette barrels and plastics are manufactured for prolonged exposure to UV light, while others may not withstand the exposure. Check with the manufacturer of those products.
4. Do not for any reason defeat the safety switches that prevent the UV light from operating with the doors open.
5. Over long periods of exposure to UV light, the plastic will begin to show signs of yellowing. This is a gradual degradation, and testing shows it to become visible only after many months of chamber use.
6. **WARNING:** This is a non-ventilated, circulation-free enclosure. It is not suitable for pathogenic, toxic, or volatile procedures. Because there is no exhaust system in this enclosure, continuous flame or high heat burners must not be utilized.
7. **WARNING:** Do not clean with alcohol or other similar cleaning solutions. See Page 8 for cleaning instructions.

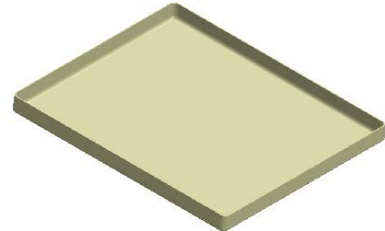
COMPONENTS

The COY CleanSpot is composed of 4 pieces. Upon receipt, the pieces should be unpacked and examined for damage. The components are described below.



A) BASE TRAY

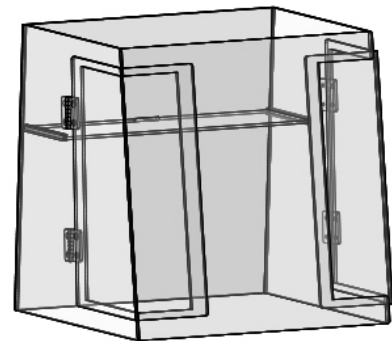
Dimensions: 1.5" high, 24" wide, 18" deep
Material: ABS plastic
Feature: Easily removed for cleaning



B) BACK & SIDES

Dimensions: 22.74" high, 52" long
Material: Acrylic
Features: As an added safety precaution, wipe down the inside of the CleanSpot with a very moist cloth prior to irradiation.

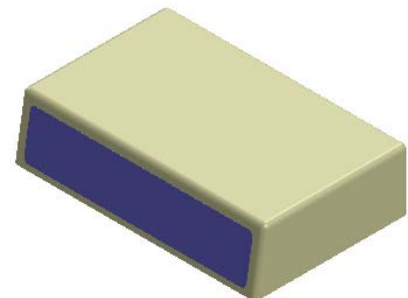
- One piece construction
- Opening for electrical cord
- Shelf
- 2 hinged doors on each side for easy interior access
- Safety switches prevent operation of UV lights with doors open



C) TOP

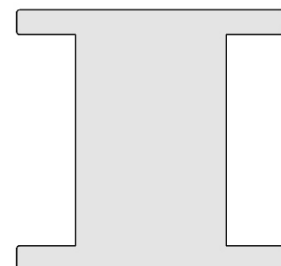
Dimensions: 5.5" high, 24" wide, 14" deep
Material: ABS plastic

- Houses the UV and white lights
- On/Off switch
- Timer switch



D) FRONT

Dimensions: 22.75" high, 14" wide
Material: Acrylic
Features: Positioned to allow maximum visibility



ASSEMBLY

To remove individual pieces, simply pull straight out of packaging. Refer to the cover and page 4 for component information and illustrations.

1. Position the Base Tray with the angled front toward you.
2. Place the Back and Side Piece inside the Base Tray.
3. The Front section should be inserted into the Base Tray with the cut outs positioned to the left and right and the beveled side toward the front.
4. Remove bulbs from the box and install. Install the white light in the center position and the 2 UV lights in the outer positions. Insert bulbs and rotate clockwise until tight.
5. Place the Top over the Back, Sides, and Front. Be sure it fits down securely, using the locator tabs.
6. For each door, plug the safety switch into the jack located in the Top Piece.
7. If you wish to use the shelf, Slide the shelf into the slots on the side.



OPERATION

FOR USE IN CONTROLLING PCR CONTAMINATION

Have all of your necessary supplies and equipment reaction set up in the chamber and leave them there during exposure to help "decontaminate" them.

1. In the CleanSpot, prepare samples containing 10X Buffer, Mg⁺⁺, distilled water, and nucleotides. Place tubes in microtube rack, uncapped.
2. Prepare biological control and place in microtube rack. This should be run with each irradiation. See BIOLOGICAL CONTROL for more information.
3. For optimal results, place microtube racks in the center of the base tray away from the shelf. You can also put samples on the shelf and use a reduced exposure time.
4. Shut the doors. The safety circuit becomes complete only when the doors are closed. By opening the doors, the UV lights are disengaged.
5. The ON/OFF switch operates the fluorescent light.
6. The TIMER operates the UV light. Set timer for 30 min. if the samples are on the base, or for 15 min. if on the shelf.
7. While still in the chamber, add the remainder of the reaction components to the tubes.
8. Cap the tubes and put them in your thermal cycler.
9. Leave all of your equipment and supplies in the chamber with the doors closed. so that they will be "clean" and ready for the next use.

NOTE: Even though you are working inside the chamber, proper handling techniques should be observed. For instance;

- don't handle or re-use tips
- always use displacement pipettes
- clean gloves

Particular care should be given to the target DNA, primers and DNA polymerase, since typically these can not be irradiated. Do not open these component tubes outside of the chamber and be sure not to contaminate them with "dirty" tips. Also avoid placing the chamber where there will be strong air currents through the chamber when the doors are open, as this will defeat the protection afforded by the still air environment.

CLEANING

1. Remove any waste or absorbent materials used in the workstation and discard appropriately.

For containment use

1. **DO NOT USE ALCOHOL.** Alcohol dries plastic, forming cracks which may affect structural integrity.
2. **DO NOT WIPE DOWN WITH DE-IONIZED WATER.**
3. If desired, the workstation can be disassembled and wiped more than thoroughly. Be sure the inner wall surfaces are completely dry before turning on the UV lights.
4. An infrequent wipe-down with a 1% (by volume) Lysol solution will not damage the acrylic. Be sure the inner wall surfaces are completely dry before turning on the UV lights.

For the reflector

1. Do not use any abrasive materials.
2. Use air or soft brush to clean the reflector. Scratches may be caused by applying pressure while using a tissue.

BIOLOGICAL CONTROLS

To assure the time of irradiation is proper, biological control(s) should be run with samples. This control should contain 10 ng of target re-suspended in your standard reaction volume using H₂O or buffer. After irradiation, the Biological Control should be amplified, using your normal cycles of PCR. There should be no evidence of amplification when μ l is electrophoresed on an agarose gel.

A typical reaction contains:

1. 10X Reaction Buffer
2. Mg⁺⁺
3. Nucleotides
4. Distilled water
5. Primers
6. Target DNA
7. DNA polymerase

Reagents 1 through 4 should be irradiated. The targets, primers and polymerase may be damaged by irradiation.

REFERENCES

1. Erlich, H.A., Gelfanel, D. and Sninsky, J.J. (1991) *Science* 252: 1643-1650.
2. Sarker, G. and Sommer, S.S. (1991) *Biotechniques* 10: 590-594.
3. Haseltine, W. (1983) *Cell* 33: 13-17.
4. Sarkar, G. and Sommer, S.S. (1990) *Nature*, 343: 27
5. Sarkar, G. and Sommer, S.S. (1990) *Nature*, (347) 340-341

FLOURESCENT SYSTEMS TROUBLESHOOTING

While normally the ballast is the first component of a lighting fixture to be checked in case of trouble with more than one lamp, as often as not the trouble lies elsewhere.

The purpose of this section is to assist in locating the source of trouble which causes a particular condition or symptom.

By following this trouble-shooting guide, you may be able to locate the source of trouble in minimum time and expense. While the guide lists may common system problems, it is presumptuous to assume that it covers all possibilities.

LAMP WON'T START	
POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) Lamp failure	1) Replace Lamp
2) Poor lamp-to-lamp holder contact	2) Twist lamp in socket, reset
3) Incorrect wiring	3) Refer to wiring diagrams
4) Low voltage supply	4) Measure voltage supply with lamps on
5) Dirty lamps or lamp pins	5) Clean surfaces on lamps and pins
6) Fixture not grounded	6) Ground fixture
7) Defective ballast	7) Replace ballast

AUDIBLE BALLAST	
POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) Loose fixture covers, panels, or other parts	1) Tighten or wedge parts
2) Ballast not mounted	2) Secure ballast to fixture
3) Defective ballasts	3) Replace ballast

VERY SLOW STARTING	
POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) Input voltage too low	1) Measure input voltage with lamp on
2) Inadequate lamp/starting-aid strip (applies only to rapid start and trigger start circuits)	2) Put lamp with 1/2" of starting plane (metal "ground")
3) Poor lamp-to-lamp holder contact	3) Twist lamp in socket, reset
4) Defective lamp	4) Replace lamp
5) Improper circuit wiring	5) Refer to wiring diagram
6) Improper ballast application	6) Check Lamp/Ballast Selection Tables
7) High humidity	7) Control humidity
8) Bulb-wall temperature too low or too high	8) Thermal test lamp at mid point, refer to lamp manufacturer data
9) Dirty lamp surface	9) Clean all surfaces
10) Defective ballast	10) Change ballast

SHORT LAMP LIFE

POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) Improper voltage	1) Measure voltage supply with lamps on and refer to Manufacturer Voltage Table
2) Improper wiring	2) Refer to wiring diagrams
3) Poor lamp-to-lamp holder contact	3) Twist in socket, reset
4) Extremely short duty cycles (many lamp starts per day)	4) Check with lamp manufacturer on lamp life data
5) Defective lamps	5) Replace lamps
6) Defective ballast	6) Replace ballast

LAMP FLICKER (SPIRALING OR SWIRLING EFFECT)

POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) New lamps	1) Should be operated 100 hours for proper seasoning
2) Drafts on lamp from air conditioning system (lamp too cold)	2) Shield lamps from air current, sleeve lamps
3) Defective lamps	3) Replace lamps
4) Improper voltage	4) Measure supply voltage with lamps on and refer to Manufacturer Voltage Table
5) Defective Ballast	5) Replace ballast

EXCESSIVE BALLAST HEATING (over 90° C case temp)

POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) Voltage too high	1) Measure input voltage with lamps on and refer to Manufacturer Voltage Table
2) Improper wiring or installation	2) Refer to wiring diagrams
3) Poor lamp maintenance (instant start and preheat systems)	3) Replace lamps
4) Wrong lamp types	4) Refer to Lamp/Ballast Selection Tables
5) Wrong number of lamps	5) Refer to Lamp/Ballast Selection Tables
6) Defective ballast	6) Replace ballast

BLINKING

POSSIBLE CAUSES	CORRECTIVE MAINTENANCE
1) High voltage	1) Measure input voltage with lamp on
2) Improper wiring or installation	2) Refer to wiring diagram
3) Poor lamp maintenance (instant start and preheat systems)	3) Replace lamp
4) Wrong type lamps	4) Refer to Lamp/Ballast Selection Tables
5) Wrong number of lamps	5) Refer to Lamp/Ballast Selection Tables
6) High ambient temperature	6) Check ambient-refer to Lamp Manufacturer Data
7) Defective ballast	7) Replace ballast

WARRANTY

COY warrants the CleanSpot (except fluorescent and UV bulbs) against defects in material and workmanship, under normal use, for 6 months from the date of receipt by the purchaser. This warranty excludes damages resulting from misuse, carelessness, or neglect. COY liability under this warranty is limited to the repair of such defects or the replacement of the product, at its option, and is subject to receipt of reasonable proof by the customer that the defect is embraced within the terms of the warranty.

This warranty is in lieu of any other warranties or guarantees, expressed or implied, arising by law or otherwise. COY makes no other warranty, expressed or implied, including warranties of merchantability or fitness for a particular purpose. Under no circumstances shall COY be liable for damages either consequential, compensatory, incidental, or special, sounding negligence, strict liability, breach of warranty or any other theory, arising out of the use of the product listed herein.