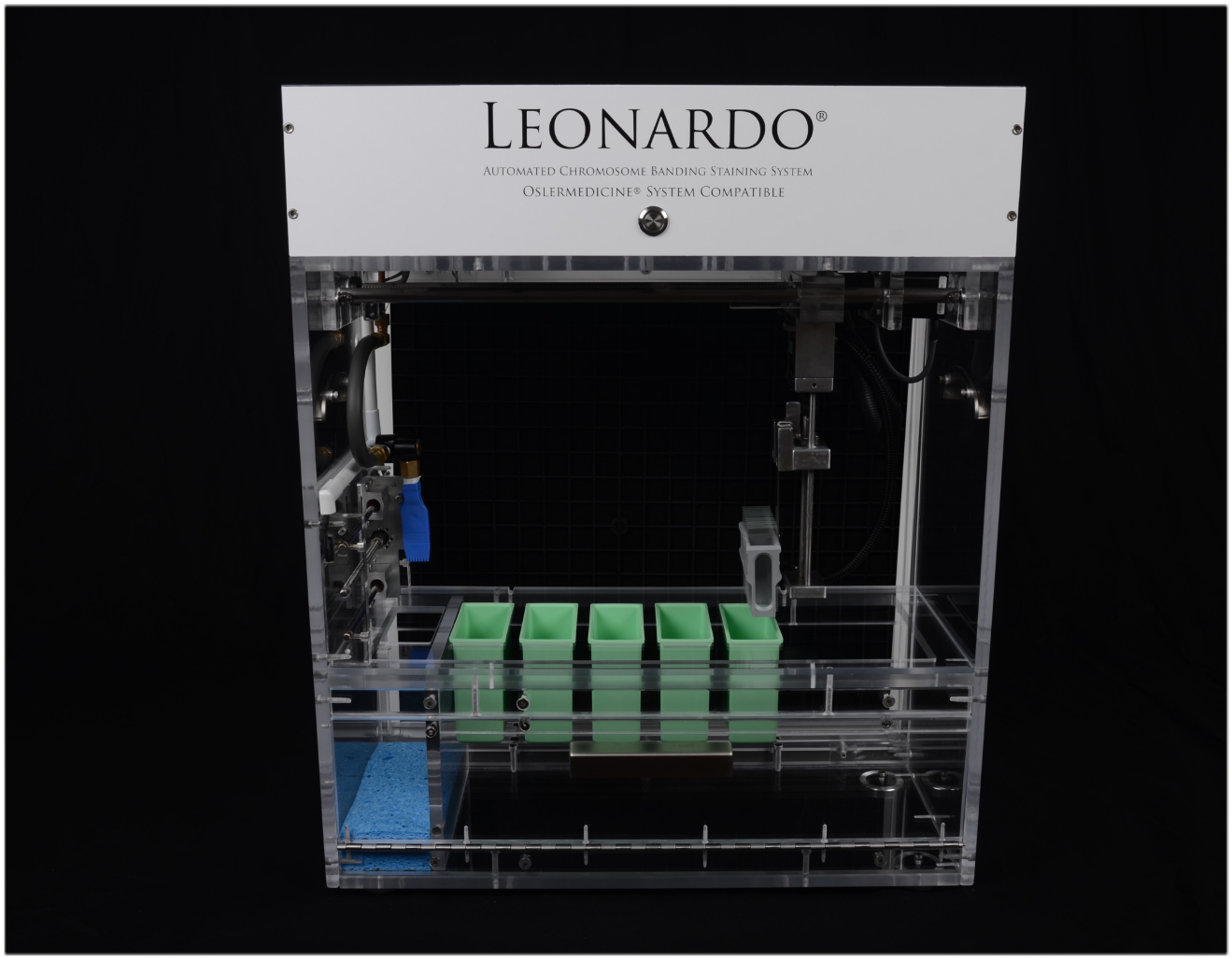

Leonardo® - Automated Chromosome Banding Staining System

UNPACKING & INSTALLATION

Leonardo® has been carefully packed in containers/boxes specifically designed for safe transportation.

Please read and follow these instructions carefully:

1. Examine the boxes for any visible damage. Please document any damage and take pictures accordingly. Serious damage to any package/box/container should be immediately reported.
2. Review the external surface of the box and locate the Phillips-head ("crosshead") screws. Remove all the Phillips-head screws from each side and then carefully remove the panel. Repeat this procedure on the opposite side (front and back). These are the only screws that you will have to remove, as the other screws used have a square center and may remain affixed.
3. Remove the top cover
4. Leonardo® is now ready to be removed from the base of the container
5. Leonardo® is heavy and will require a table or bench that can support it safely and securely. Additionally, the space allocated should be sufficient to hold the included computer, and clearance should be available for the brass fitting that protrudes from the rear of the unit (on the left side).
6. Remove the plastic covering
7. Remove all the accessories and parts that are included inside of Leonardo®
8. Once that the location has been determined, have TWO individuals lift the unit by grasping handles located at either side and lift from the base. **Caution: Do NOT attempt to have one single person lift Leonardo®**
9. Place it securely on a bench or table
10. Next, find the counterpart bronze fittings for the compressed air connection. It has two components: one that is screwed into the bronze outlet and a connector at the left back side of the instrument. Use the included Teflon® tape to fully wrap the thread, and carefully screw the fitting, tightening it securely with the [included] wrench. Once it is tight, level the connector slightly so that it remains horizontal after tightening.
11. The other related component to this is the *female* plug-in that should connect to the compressed air supply source. Two compression sleeves have been included; only one is needed to affix the connector to the hose. The *spare* is included in the event it needs replacement.
12. Leonardo® has a filter that retains particles and organic chemicals to prevent contamination of the room where it operates. This filter may need replacement, depending upon the amount of reagents used. An additional filter is included, and others may be ordered directly from elja®.
13. Leonardo® operates under "negative air pressure" over the working area where the operator works with the objective of minimizing chemical fume exposure. By design, the fans that create the negative pressure CAN NOT be turned off, and power up in conjunction to the main power switch.
14. Confirm that the filter is installed
15. Unpack the computer and connect the power supply to a 110V source, and to the computer
16. Connect the USB cable to the computer as indicated by the labels, and then to the back of Leonardo®.
17. Connect the 110V power cable to the back of Leonardo® and then to the power outlet in the wall (or surge protector if so provided).
18. Power ON Leonardo® by pressing the silver switch located in the middle of the top panel that bears the Leonardo® name. A blue light should come on.
19. Power the computer ON, and wait for the main screen of Leonardo® software program to display.

20. THE ABOVE PROCEDURE SHOULD BE FOLLOWED ROUTINELY: *TURN LEONARDO® ON FIRST AND THEN POWER ON THE COMPUTER.* This will alleviate the possibility of the computer and Leonardo® not communicating and thus subsequently generating an error message.
21. Place the containers (green or clear may have been shipped) in the tray, (extra containers have been included for your convenience). The last station does not require a container, as you will place the included sponge at the bottom.
22. CAUTION: At this point of the setup - **DO NOT YET PLACE THE SLIDE CARRIER ON THE MOVING AXIS**
23. If for any reason you need to stop Leonardo®, press the ESCAPE key, and this will halt the current protocol.
24. If you are ready to test Leonardo®, simply read the menu on the screen, select “Run a Protocol”.
25. Choose “Banding without Split”, and click on this option.
26. As a result of this selection, Leonardo® will take the following actions:
    a. move the Z-axis “HOME”
    b. move to station 1, and move down
    c. begin to agitate
    d. Leonardo® will stop and move up and to the next station
    e. descend and start agitation
    f. stop, move up, and go to the third station
    g. descend and start agitation
    h. stop and move to the next station
    i. descend and agitate
    j. stop and move to the drying station
    k. the air should be automatically switched on when the Z-axis arrives to this position
    l. the Z-axis will descend and stop.
    m. the Y-axis will begin from the back to the front and vice versa until the time programmed for drying has passed.
    n. the Z-axis will move up and then return home.
27. Leonardo® has been calibrated on all axes prior to shipping and should not require any further adjustments
28. Install one of the included slide holders: Simply “hang” it in place on the Z-axis, and it should slide into its resting position
29. Run the same protocol as in #25 (above)
30. In the event that Leonardo® is out of calibration, the slide carrier will not go into the container at a given station and it will dislodge from the Z-axis mechanism, remaining outside of the station’s container. This is a failsafe mechanism in order to prevent slide damage.
31. If you have completed the previous steps without issue, Leonardo® is now ready for regular use.
32. The protocols included may be used, or you may create those of your choosing

CALIBRATION (NOT REQUIRED AFTER SHIPPING)

1. As previously stated, Leonardo® is shipped fully calibrated on all axes and does not require any active adjustment in the majority of cases. In the event that you feel the need to make changes, please begin by selecting the "Utilities" menu from the software.
2. Select "Calibrate" (Found in the "Utilities" menu)
3. From the "Calibrate" menu, there are three choices:
   I. X-Axis, moves from left to right
   II. Y-Axis, moves front to back
   III. Z-Axis, moves up and down
   For further clarification: **The following Axes are labeled on Leonardo®:**
   *The X-axis is the long axis of the system.
   *The Y-axis is the up/down distance of the system, the depth of the containers.
   *The Z-axis is the left side axis which moves front to back in the drying section.
4. Place the slide carrier, WITHOUT SLIDES on the Z-axis. Make sure that it is properly seated.
5. Note: Container 1 is furthest to the right, while container 6 is the furthest to the left.
6. To calibrate the X-axis, use the Option menu: move the X-axis using the left arrow to align it perpendicular and at the center of container 1, using the up and down arrows, be certain that the slide carrier enters and leaves the container freely without touching the walls of the container or Y-axis. If satisfied, press the button SAVE. The position of the slide carrier in relationship to the position of container 1 has been calibrated and saved.
7. Do the same for the other container and for the drying station that does not have a container.
8. The calibration for the up and down position for the slide carrier is done using the up and down keys. You decide the correct position, the slide should not go so deep that the label is submerged into the reagents, but it should go deep enough so that the chromosome preparations are completely submerged into the reagent.
9. Once the container is selected, use the appropriate arrow keys (right or left) on the keyboard to move the gantry. The slide carrier will be calibrated to the appropriate container once it is positioned correctly. Once the correct position is found, then click the SAVE button to save the location for that container. It is best to start at container 1, then 2, 3 and so on.
10. The calibration for the Z-axis at the drying station is different from the others, because the slide carrier is not submerged as deep as it is in the other stations (containers), it is placed about ¼ of an inch below the edge of the blue wand that distributes the compressed air. This is done to use the compressed air at its highest efficiency to dry the slides.
11. The Y-axis (the one that moves front to back and the one that has the blue wand for the compressed air) is to be calibrated so that it moves ½ inch from the back and ½ inch from the front of the slides, to allow for them to be fully covered by the compressed air and to dry completely and quickly. Leonardo® was calibrated before shipping and it is very likely that it does not need to be recalibrated.
12. Please remember that the appropriate preparation of chromosome bands of high quality and resolution requires preparations of high quality, and new reagents of the highest purity and quality and that avoiding reagent cross contamination needs to be implemented. The procedures programmed in Leonardo include a pause for the reagents to drain before moving to the next station (reagent).
13. Once that you have used Leonardo®, please explore the other options in the menus of the program including the creation of your own PROTOCOLS, that are composed by PROCEDURES that are composed by STEPS that are listed in the appropriate menu.

DESCRIPTION & BACKGROUND

Leonardo®

Leonardo® has been designed to perform the following functions:

- Move slides from one reagent to another (reagents are in containers held inside of the Leonardo® tray)
- Submerge and remove slides from reagent containers for the times specified by the user
- Agitate slides inside the reagent at a speed and time specified by the user
- Automatically dry slides using compressed air (DryWand™)

Disclaimers:

- No other action, function(s), or effect(s) are offered or implied as a capability or use of Leonardo®
- The responsibility for writing protocols and use are the unique responsibility of the user
- elja® provides three protocols that have been tested and validated by an independent reference laboratory and have been demonstrated to produce bands of excellent quality with the reagents that were used. This is not indicative of such warranty or a promise that the user will have the same outcome.
- Leonardo® is constructed with high impact Plexiglass®, stainless steel, and aluminum. As with most any materials, they can be damaged by improper use and by solvents. Damage due to improper use is not covered under warranty.

Background

Leonardo® is an instrument that is designed to hold and to move slides from one container to the next, and to hold slides inside a container for a period of time with or without agitation. It allows for manual staining techniques to be standardized and automated, which can dramatically improve results.

1. Leonardo® has a number of stations that correspond to those most commonly used in chromosome banding protocols
2. Leonardo® has the option of accommodating an alternate number of slides by installing (optional items) a corresponding tray, slide holder, and reagent container:
    1. Slide holders are available in max capacities of 4x, 8x, and 24x
    2. **Your shipped Leonardo® includes 2x trays, containers, and 3x slide holders with a maximum capacity of 4x slides. This is a flexible option as it allows a lab to use small volumes of reagents, while still maximizing automation flexibility.**
3. Leonardo® is operated by executing a computer program that allows the user to program:
    a. Protocol (e.g. G banding with Giemsa for peripheral blood)
    b. Procedure (e.g. trypsinization of the slides)
4. c. Steps of each Procedure (e.g. bring down the slide holder into the trypsin container, agitate, remove the slide holder from the trypsin container, advance to the next procedure)
    d. The time that is allocated to each one of the steps for each procedure of each protocol.
5. The user may program multiple protocols for different banding and staining procedures and execute them independently
6. Using the most common protocols for G banding, the total time for the completion of one protocol is less than 20 minutes. If the time needed for the set up of the instrument is taken into account, it is estimated that the

instrument can run a protocol every 30 minutes, and if the laboratory operates during eight hours, the instrument can be used to process more than 10 batches of slides of banding per day.

7. The definition of the protocols and procedures, and of their steps is controlled by the operator, who determines the times respectively of each.
8. One of the most important procedures in the processing of slides for cytogenetic analysis is the washing and drying of slides. The last (large) container (located on the **left** hand side of Leonardo®) should contain enough water to remove all the Giemsa left over the slide. The next station, the drying (DryWand™) station, uses compressed air to rapidly dry slides and remove any fluid that may dry create marks and/or discoloration on the chromosomes. It is recommended that the compressed air be set to 30 lb/square inch. **The regulator is located at the upper left, back side of Leonardo®.**
9. Prior to shipping, Leonardo® was set to 30 lb/sq inch. The brass connector should be connected to a *clean* compressed air source, so as to not damage the slides. The compressed air is evenly applied over the slides when they arrive at the DryWand™ drying station.
10. PLEASE NOTE: **DO NOT OPEN THE ELECTRONICS COMPARTMENT AT THE TOP OF THE CHAMBER UNLESS YOU ARE FOLLOWING SPECIFIC INSTRUCTIONS PROVIDED TO YOU BY elja®.**
11. The use of Leonardo® requires that one operator write the protocol, load the slides, remove them after the completion of the protocol, fill the containers with the reagents, remove and wash the containers. The constancy of the times for each one of the steps of the procedure of each protocol, and the constancy of the times between different procedures and of the agitation, make Leonardo® a very reproducible method for chromosome banding.

TIPS

It is important to recognize that the quality of bands of a chromosome depends greatly on the quality of the metaphases; they should:

1. Be free of cytoplasm
2. Have long and straight chromosomes with few or no overlaps
3. Be dry to produce a degree of humidity of a gray (not black or bright pattern) under phase contrast microscopy
4. Be properly “aged” after spreading and prior to banding

Chromosome band formation takes place in three steps:

1. The hypotonic, the fixatives and their effects
2. The preparation of the slides, proper drying time, spreading and water retention. **When using Monalisa® this step is greatly facilitated and becomes highly reproducible.**
3. The process of trypsinization, buffering, and staining which is facilitated by the use of Leonardo®

The quality of the reagents used during the above processes plays a critical role in the formation and final appearance of the chromosome bands.

****If you have any problems or concerns please contact us at: solutions@eljainc.com**