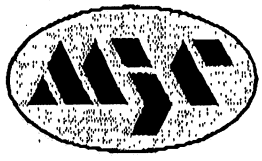
Twin Tower™ Block
Operations Manual



Suppl. 3 to *DNA Engine Operations Manual,* V 2.0

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Safety Warnings

Warning: The Twin Tower's sample block can become hot enough during normal operation to cause serious burns if touched or to cause liquids to boil explosively. Wear safety goggles or other eye protection at all times during operation.

Caution: Never remove the Twin Tower block from the PTC-200 DNA Engine™ or PTC-225 DNA Engine Tetrad™ with the power turned on. Doing so can cause electrical arcing that can melt the contacts in the connector joining the Twin Tower block to the PTC-200 DNA Engine or PTC-225 DNA Engine Tetrad.

Packing List

After unpacking the Twin Tower block, check to see that you have received the following:

- Twin Tower block
- *Twin Tower Block Operations Manual* (this document)

If either of these items is missing or damaged, contact MJ Research or the authorized distributor from whom you purchased the Twin Tower block to obtain a replacement. Save the original packing materials in case you need to return the Twin Tower block for service. See the *PTC-200 DNA Engine Operations Manual* (V 2.0) for shipping instructions.

Description

The Twin Tower block is the latest addition to the Alpha™ unit series of interchangeable sample blocks for the PTC-200 DNA Engine and PTC-225 DNA Engine Tetrad. The Twin Tower block consists of two independently controlled programmable sample blocks that provide oil-free thermal cycling for slides. Each sample block consists of a vertical metal rack with slots for up to 16 standard 25- x 75-mm slides.

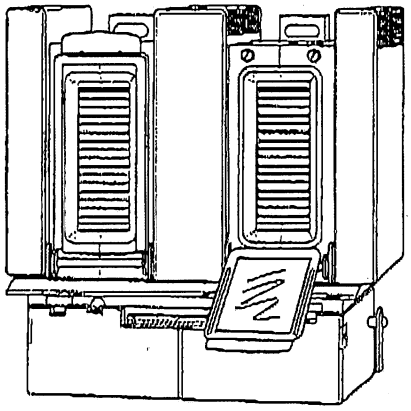
A clear acrylic front door and a removable sliding back plate provide access to the interior of each sample block for loading and removing slides and for cleaning slots. Slides heat and cool uniformly as long as there is complete contact between their bottoms and the metal slots.

The Twin Tower block is available in two configurations:

- **Front-facing,** with doors opening toward the front (as in fig. 1), for use in the DNA Engine; can also be used in the DNA Engine Tetrad, but the doors will not open completely, so slides must be loaded from the rear of the unit.
- **Reversed,** with doors opening away from the front (not depicted), available only for the DNA Engine Tetrad; allows the doors to be completely opened when the unit is installed.

A wide variety of reactions done on slides may be run in the Twin Tower block, such as PRINS, RT-PRINS, *in situ* PCR* and RT-PCR, and immunohistochemical procedures. The Twin Tower block may also be used to perform both the denaturation and humidified incubation steps of *in situ* hybridization, eliminating the need for a separate humidification chamber.

Figure 1 The Twin Tower, front view.



Specifications

- **Thermal range:** 4°–105°C, but no more than 23°C below ambient temperature.
- **Accuracy:** ±0.4°C at 90°C, NIST-traceable.
- **Thermal homogeneity:** ±0.6°C after 60 sec of arrival at 90°C; ±0.3°C after 30 sec of arrival at 60°C.
- **Ramping speed:** Up to 1.2°C per sec.

Installation

See the *PTC-200 DNA Engine Installation Guide* for instructions on situating the PTC-

* The polymerase chain reaction (PCR) is a process covered by US patents #4,683,195 and #4,683,202, which are owned by Hoffmann-La Roche. Users must obtain a license to perform the reaction. A license is currently available through either Roche Molecular Systems of Branchburg, New Jersey, or Perkin Elmer of Norwalk, Connecticut.

200 DNA Engine, which will help ensure proper performance of the Twin Tower block. Note that air circulated across the block's heat sink exhausts from vents at the *top* of the block.

Mount the Twin Tower block into the DNA Engine in the same way that other Alpha units are mounted (see the *PTC-200 DNA Engine Operations Manual*).

Operation

Required Software Version

PTC-200 DNA Engines with software version 1.1K or higher and PTC-225 DNA Engine Tetrads with software version 1.2D or higher can operate the Twin Tower block. To determine the software version number of either machine, go into the Setup Menu and select *Version*. Free software upgrades are available from MJ RESEARCH.

Preparing Slides

The Twin Tower block can accept slides made of any thermally conductive material and of any shape as long as the finished slides fit within the slots.

To ensure that all samples reach the same temperatures during thermal cycling, the bottoms of loaded slides must be in complete contact with their metal slots. Do not use any slide preparation method that would interfere with this, such as clips that extend under the slide. Do not use mineral oil under slides to enhance thermal contact. This is unnecessary, and the oil is difficult to completely clean off, eventually making the slots sticky.

Use special care in sealing cover glasses or the edges of two-slide preparations (i.e., where a slide is being used as a cover glass) with such sealers as nail polish or rubber cement. Allow the sealer to completely dry before loading slides into slots, to avoid getting sealer on the surface of the slots (especially a problem with two-slide preparations). Keep the bottoms of slides free of sealer, to avoid interposing anything between the slides and the bottom of the slide slots. Self-Seal™ reagent and Frame-Seal™ incubation chambers, available from MJ RESEARCH, are effective alternatives to mechanically sealing slides (see p. 7). Other approaches to slide sealing are possible; contact MJ RESEARCH for more information.

Loading Slides

1. Open the door to one of the sample blocks by pulling downward on the latch at its top. When using a front-facing unit in the DNA Engine Tetrad, remove the back panel to one of the sample blocks by pulling upward on it.

2. Inspect the sample block to ensure that its slots are clean. Clean slots as needed before loading slides (see pp. 5–6).

3. Place slides in slots. Avoid dislodging unsealed cover glasses or the top slides in two-slide preparations. Using a forceps can make it easier to load slides

   Note: Make sure all slides are lying flat in their slots. Remove slides that are not lying flat, clean off or remove whatever is interfering with the slides' seating, and replace the slides in the sample block.

4. Close the door (or slide the back panel down). Make sure that the back panel is completely closed, to ensure that samples heat and cool uniformly.

Running a Protocol

Both the PTC-200 DNA Engine and PTC-225 DNA Engine Tetrad can operate the Twin Tower block. Protocols are started on each sample block individually.

The Twin Tower block can run protocols using calculated control or block control. Probe control is not available because there is no in-sample probe for this block. See the *PTC-200 DNA Engine Operations Manual* for a complete explanation of these temperature control methods.

Note: Use calculated control whenever possible, since it delivers more precise control over sample temperature than the block-control method.

Using Calculated Control

1. Select a calculated-control protocol, then press «Proceed» ("Quikstep" has been chosen in the example). A screen identifying the first available sample block (*A* in the example) will be displayed:

```
Run: QUIKSTEP  on  A


Block: 16S(L)
```

2. Press «Block» to select a sample block to run the protocol on. The status indicator light for the selected block will flash green. With the proper sample block selected, press «Proceed». The slide format menu will be displayed:

```
Run: QUIKSTEP    on A

Enter Slide Format
_SINGL  Doubl  Mass
```

Singl indicates a slide and cover glass preparation; *Doubl* indicates a two-slide preparation. Select either of these options to enter a predetermined average mass into the program's calculation of sample temperature. Select the *Mass* option to enter the exact mass of a particular slide format. *Mass* provides the most accurate temperature control of the three options because an exact value for the loaded samples is used in the calculation of sample temperature.

3. Select *Singl*, *Doubl*, or *Mass*, then press «Proceed». If *Singl* or *Doubl* is selected, the protocol will begin running, and the runtime screen for the protocol will be displayed. The sample block's status indicator light will change from flashing green to a constant red or a constant green, depending on whether it is heating up or cooling down.

   If *Mass* is selected, a screen similar to the following one will be displayed:

```
Run: QUIKSTEP    on A


Mass (g): 5.0
```

   Use the keypad to enter the mass in grams of a single fully assembled repre-

sentative slide (from 0.1g to 13.0g). Press «Cancel» to clear a value from the screen and start over.

Press «Proceed» when the correct mass has been entered. The program will begin running, and the runtime screen will be displayed.

4. To run a protocol on another sample block, press «Block» to display the Main Menu.

   - **DNA Engine:** Choose a protocol and provide the information requested by the slide format menu, as for steps 1 and 2. It is not necessary to select a block because the unused block will automatically be chosen.
   - **DNA Engine Tetrad:** Repeat steps 1 and 2, pressing «Block» until the desired block is selected.

   Press «Block» to see the runtime screen for each active block. As you press this key, the display will cycle from the Main Menu to the runtime screen for each active block, then back to the Main Menu. As the runtime screen for each block displays, its status indicator light flashes briefly and then returns to a constant glow.

Using Block Control

1. Select a block-control protocol, then press «Proceed».
2. Select a sample block (see step 2, above), then press «Proceed».

No special information must be entered, since block-control programs simply heat or cool the sample block to the temperatures specified in the protocol.

Adapting Programs

MJ RESEARCH has established that block-control protocols developed for the PTC-100-16MS Slide Block™ generally can be used without modification on the Twin Tower block. To convert any of these protocols to calculated control, shorten all incubations by 20 seconds. Some empirical testing may be required to fine-tune the protocols.

Performing *In Situ* Hybridization

The Twin Tower block can be used as a humidified chamber for steady-state incubations (e.g., hybridizations, color-development reactions). To humidify a sample block for an *in situ* hybridization, push one laboratory tissue into the bottom slot and inject 1ml of deionized water onto it. This should provide ample moisture for the entire sample block. Reapply 1ml of deionized water to the tissue for successive reaction runs. Remove the tissue following the last reaction run, and blot up any water visible on the inside of the sample block.

Maintenance

Remove the Twin Tower block from the PTC-200 DNA Engine or PTC-225 DNA Engine Tetrad before cleaning it, to prevent fluids from entering the machine's chassis and causing electrical shorting. Use only the methods described in this supplement to clean the machine.

Clean the outside surfaces and doors of the Twin Tower block with a mild soap solution or water-based laboratory cleaner and a soft

cloth. Avoid strongly alkaline cleaners and chlorinated solvents. Ethanol (95%) may be used on all the Twin Tower's outer surfaces except the acrylic doors.

Remove extraneous materials from slide slots with water and a long-handled cotton swab, small glassware cleaning brush, or tongue depressor wrapped in laboratory tissue. Pay particular attention to cleaning the bottoms of slots. Use any of the above-named solutions if water does not work. Remove nail polish from slots with acetone. Use acetone sparingly, and do not use it on the doors.

Do not immerse the Twin Tower block in water or otherwise expose the bottom of the block to water, to avoid damaging its circuit board. If necessary, carefully blot fluids off of the bottom of the block with a soft cloth, and allow it to dry completely before use.

Troubleshooting

See table 1 to troubleshoot problems with the Twin Tower block.

Technical Support

Please contact our technical support staff by telephone, fax, or E-mail (see p. 8) if you have questions about operating the Twin Tower block or if you experience unusual reaction failures that may be related to Twin Tower block performance.

Table 1 Troubleshooting Twin Tower Block Problems

Problem	Probable Cause	Recommended Action
Display shows this error message: Unknown Block Type.	Incorrect software version is installed.	Update software to correct version for type of machine in use. Contact MJ RESEARCH or an authorized distributor.
Poor results are obtained when using glass slides and running in calculated-control mode.	Wrong sample weight was entered into protocol.	Enter correct sample weight into protocol.
Poor results are obtained when using non-glass slides and running in calculated-control mode.	Thermal characteristics of slide are too different from those of glass for machine to use calculated-control method accurately.	Use block control and fine-tune protocol empirically.
Quality of results is erratic. Some samples show good results, and others do not.	Not all slides are in complete contact with sample block.	Ensure that slots are completely clean. Follow instructions on p. 3 for preparing two-slide preparations. Ensure that slides lie perfectly flat in slots.

Appendix 1: Ordering Supplies

MJ RESEARCH offers two options for sealing slides for high-temperature incubations, Self-Seal reagent and Frame-Seal incubation chambers. When added to an *in situ* reaction mix, Self-Seal reagent (patent pending) automatically creates an evaporation-limiting barrier around the periphery of a slide cover glass. There is no need to use fingernail polish; rubber cement, or special slide-mounted reaction chambers. Self-Seal reagent is supplied as a 2X concentrate in deionized water.

Frame-Seal incubation chambers provide an alternative approach to sealing slides that is useful when it is important to recover all of the reaction mix after thermal cycling. To try both approaches to slide sealing, order the MJ RESEARCH Slide Sealing Starter Kit.

Item	Package Size	Part #
2X Self-Seal™ reagent	5 tubes, 1ml each	SLR-0101
Frame-Seal™ incubation chambers	100 frames, 25-µl capacity, 1–3 frames/slide	SLF-0201
	100 frames, 65-µl capacity, 1–2 frames/slide	SLF-0601
	100 frames, 125-µl capacity, 1 frame/slide	SLF-1201
Slide Sealing Starter Kit	2 tubes, 1ml each 100 chambers, 65-µl capacity 100 unchambered slides 100 3-chambered slides 80 covers, 24mm x 60mm (Sufficient for 80 x 50-µl reactions sealed with Self-Seal reagent plus 100 x 65-µl reactions in Frame-Seal incubation chambers.)	SLR-0201

MJ RESEARCH, INC.

Factory and Headquarters
149 Grove Street
Watertown, Massachusetts 02472 USA

Product Information: (888) 735-8437
Cust. service & support: (888) 652-9253
General fax: (617) 923-8080

West Coast Office
384 Oyster Point Blvd. #8
South San Francisco, California 94080 USA

Product information: (415) 635-1320
General Fax: (415) 635-1326

National Sales Office
987 Tahoe Blvd. #106
Incline Village, Nevada 89451

All orders: (888) 729-2165

Web site: www.mjr.com

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