Microtiter Calibration

The microtiter base plate has been designed to fit two 96 or 24 sample microtiter racks or two custom 48 sample microcentrifuge racks (Part #: 21148 from Syringa Lab Supplies) to use exclusively on the KinExA 4000 and Autosampler. KinExA Pro Software Version 4.1.6 or higher is needed to use the microtiter racks.

- Place the microtiter base plate on the instrument so that the adjustment knobs are closest to the wash station and it sits where Rack 2 would normally fit (see ***Figure 1 for Autosampler positioning & Figure 2 for 4000 positioning***).

Note: Rack 1 can be left in place however rack 3 needs to be taken out to fit the microtiter base plate.

- Open the KinExA Pro software. Under the **Tools** menu, select **Options**. In the **KinExA Pro - Options** window select **Autosampler Configuration**. Designate Rack 2 and Rack 3 as either 96 or 24 Sample Microtiter or 48 Sample Microcentrifuge (***Figure 2***). Rack 2 is now the left hand plate and Rack 3 is the right hand plate.

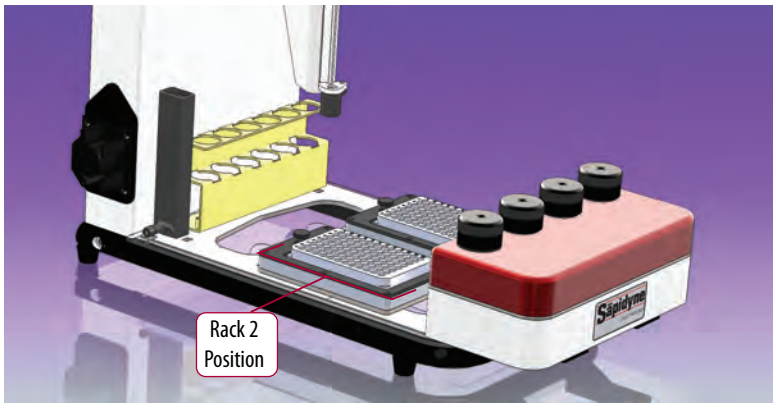

Figure 1. *Microtiter base plate in the correct position on the Autosampler.*

Figure 2 *Microtiter base plate in the correct position on the KinExA 4000*

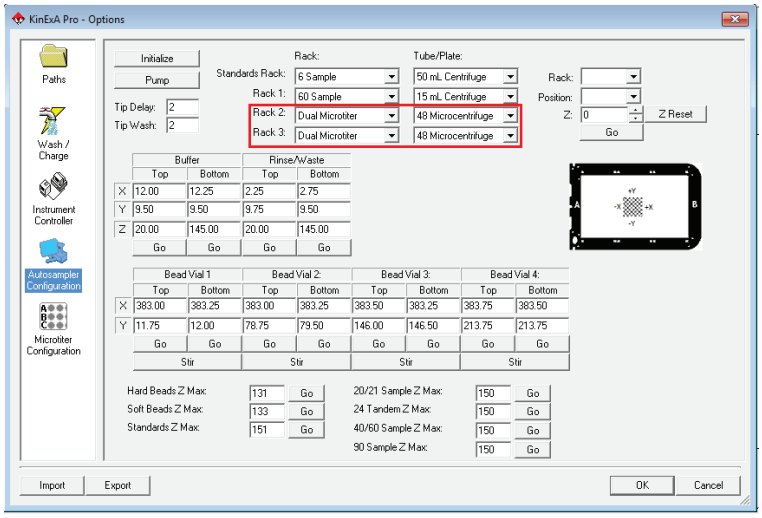

Figure 3. *Autosampler configuration screen*

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Calibrate the Left Hand Plate Positions (Rack 2)

- Place the **Microtiter Calibration Guide** in the left plate so that the intersecting yellow lines are closest to the quad stirrer (***Figure 4***).

- Select **Microtiter Configuration** below the Autosampler configuration option. Once microtiter configuration is selected, the left hand plate calibration screen will appear (***Figure 5***).

- Drive the sipper tube to position H12 by selecting the gray button in column H, row 12. (***Figure 5***) Drive the sipper tube down by entering **132** in the **Z parameter** and select **Go**.

Note: The sipper tube can be lowered so it is directly over the Microtiter Calibration Guide. The sipper tube should be close to, but not touching, the guide. Use the up or down boxes next to the Z parameter to raise or lower it.

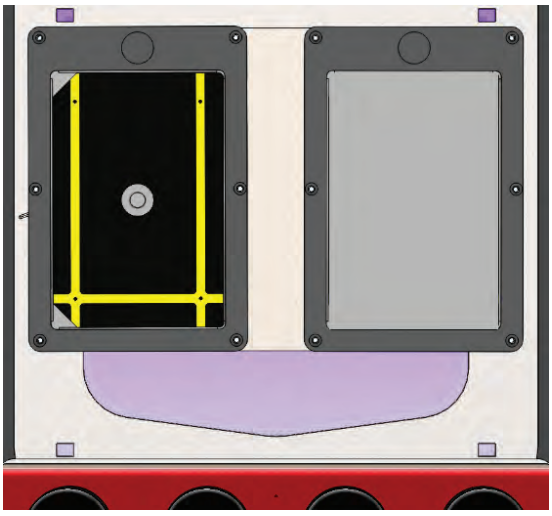
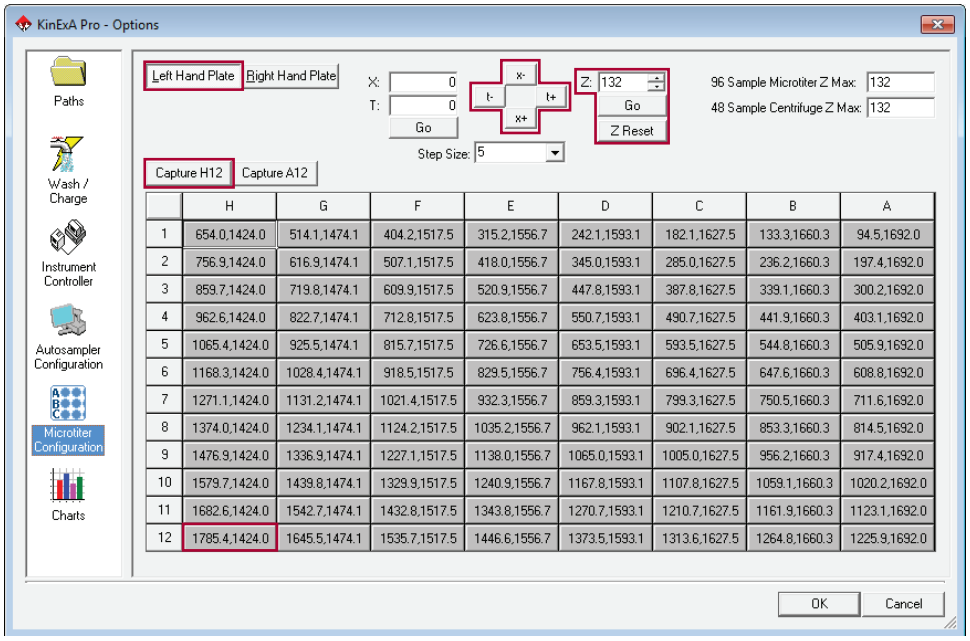


Figure 4. *Microtiter calibration guide in rack 2.*



	H	G	F	E	D	C	B	A
1	654.0,1424.0	514.1,1474.1	404.2,1517.5	315.2,1556.7	242.1,1593.1	182.1,1627.5	133.3,1660.3	94.5,1692.0
2	756.9,1424.0	616.9,1474.1	507.1,1517.5	418.0,1556.7	345.0,1593.1	285.0,1627.5	236.2,1660.3	197.4,1692.0
3	859.7,1424.0	719.8,1474.1	609.9,1517.5	520.9,1556.7	447.8,1593.1	387.8,1627.5	339.1,1660.3	300.2,1692.0
4	962.6,1424.0	822.7,1474.1	712.8,1517.5	623.8,1556.7	550.7,1593.1	490.7,1627.5	441.9,1660.3	403.1,1692.0
5	1065.4,1424.0	925.5,1474.1	815.7,1517.5	726.6,1556.7	653.5,1593.1	593.5,1627.5	544.8,1660.3	505.9,1692.0
6	1168.3,1424.0	1028.4,1474.1	918.5,1517.5	829.5,1556.7	756.4,1593.1	696.4,1627.5	647.6,1660.3	608.8,1692.0
7	1271.1,1424.0	1131.2,1474.1	1021.4,1517.5	932.3,1556.7	859.3,1593.1	799.3,1627.5	750.5,1660.3	711.6,1692.0
8	1374.0,1424.0	1234.1,1474.1	1124.2,1517.5	1035.2,1556.7	962.1,1593.1	902.1,1627.5	853.3,1660.3	814.5,1692.0
9	1476.9,1424.0	1336.9,1474.1	1227.1,1517.5	1138.0,1556.7	1065.0,1593.1	1005.0,1627.5	956.2,1660.3	917.4,1692.0
10	1579.7,1424.0	1439.8,1474.1	1329.9,1517.5	1240.9,1556.7	1167.8,1593.1	1107.8,1627.5	1059.1,1660.3	1020.2,1692.0
11	1682.6,1424.0	1542.7,1474.1	1432.8,1517.5	1343.8,1556.7	1270.7,1593.1	1210.7,1627.5	1161.9,1660.3	1123.1,1692.0
12	1785.4,1424.0	1645.5,1474.1	1535.7,1517.5	1446.6,1556.7	1373.5,1593.1	1313.6,1627.5	1264.8,1660.3	1225.9,1692.0

Figure 5. *Microtiter configuration screen.*

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- Check to make sure the sipper tube is directly over the black dot where the two yellow lines intersect. The sipper tube will be over the dot when it is in the center of the two yellow lines (***Figure 6***). If the sipper tube is not on the black dot or centered follow the instructions below to adjust the sipper tube position.

  1) Adjust the right arc or left arc by selecting the **t-** button or **t+** button (***Figure 5***).
  2) Adjust the forward or backward movement of the sipper tube by selecting the **x+** button or **x-** button (***Figure 5***).

Note: Moving the **t** parameter can change the **x** position. Look at the sipper tube from multiple angles to make sure the sipper tube is aligned in the H12 position. The step size is in mm and can be changed

- Once the sipper tube is aligned in the H12 position, select **Capture H12** (***Figure 5***).

Note: If the Z parameter is not cleared, the sipper tube will drop to this setting every time a new position is selected. To clear this setting, select the **Z Reset** button (***Figure 5***).

- Next move the sipper tube to the H1 position by selecting the gray button in column H, row 1. Drive the sipper tube down by entering the appropriate **Z parameter** and select **Go** (***Figure 5***).

- Check to make sure the sipper tube is centered in the yellow line. If not, loosen the adjustment thumbscrew and swivel the left plate until the black dot is centered under the sipper tube (***Figure 7***). Once aligned, tighten the thumbscrew.

Note: Do not change the **t** or **x** parameter as this will cause the H12 alignment to be off.

- Move the sipper tube to the A12 position by selecting the gray button in column A, row 12. Drive the sipper tube down

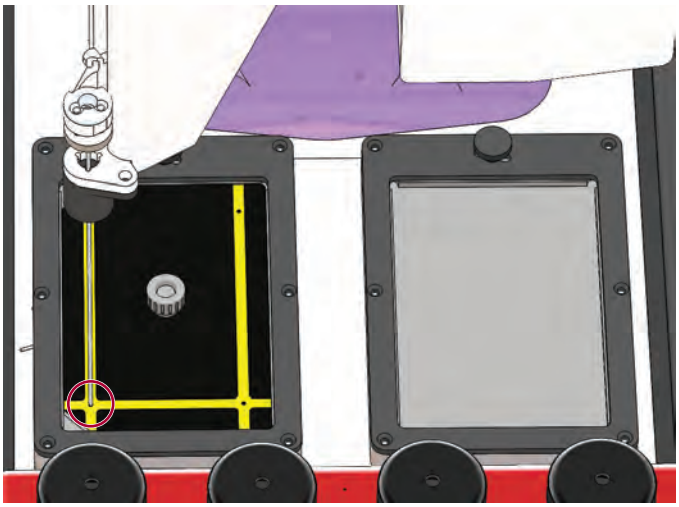

Figure 6. *Sipper tube alignment in H12 position.*

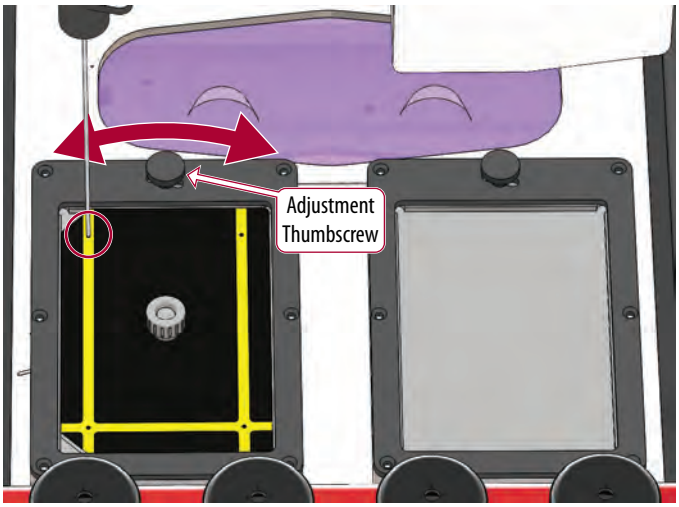

Figure 7. *Align H1 position by loosening the adjustment thumbscrew and swiveling the plate left or right.*

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by entering the appropriate **Z parameter** and select **Go**.

- Check to make sure the sipper tube is directly over the black dot where the two yellow lines intersect. The sipper tube will be over the dot when it is in the center of the two yellow lines (***Figure 8***). If the sipper tube is not on the black dot or centered follow the same instructions for adjusting the **t** and **x** parameters for position H12.

- Once the sipper tube is aligned in the A12 position select **Capture A12**.

Calibrate the Right Hand Plate Positions (Rack 3)

- Select the gray **Right Hand Plate** button in the top left hand corner of the Microtiter Configuration screen.

- Place the **Microtiter Calibration Guide** in the right hand plate.

- Perform the same procedures as the **Left Hand Plate Positions**.

Adjust the Z Max

- Once the positions are set, place the desired 96, 48, or 24 sample rack into the left hand plate. Be sure to select the **Left Hand Plate** button.

- Select the gray button in column H, row 12. Drive the sipper tube down by entering **132** in the **Z parameter** and select **Go**. Increase the Z parameter until the sipper tube just barely hits the bottom of the tube or well plate.

Note: The base plate is spring loaded to allow the sipper tube to barely hit the bottom of the tube without causing a collision error.

- Once the **H12** position is checked, check the Z parameter in the **H2**, **A1**, and **B12** positions. When finished, perform the same test for the right hand plate.

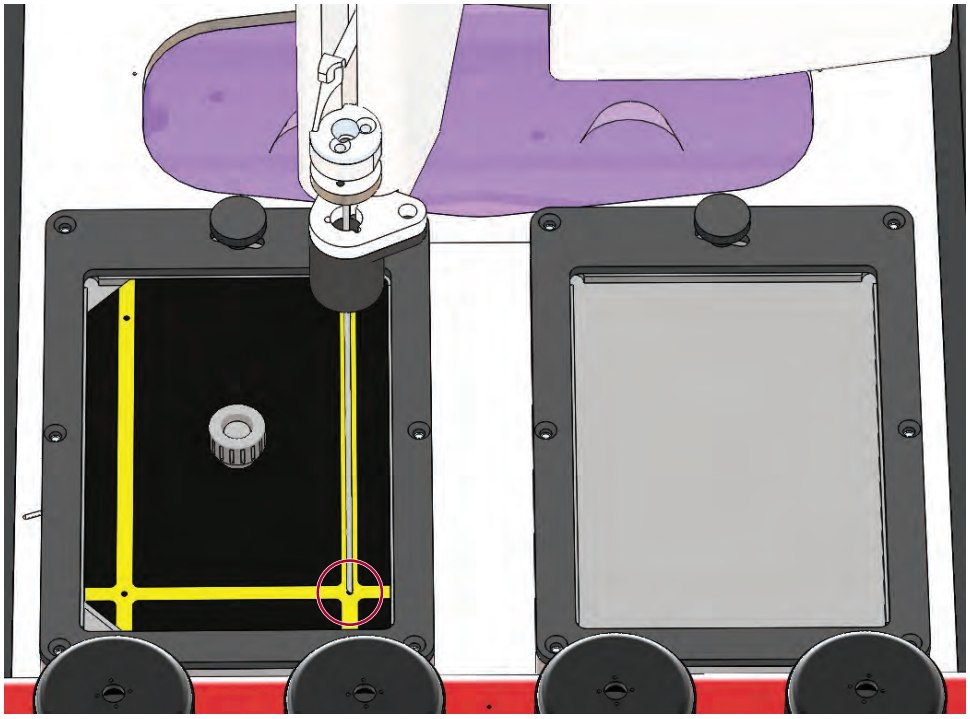

Figure 8. *Sipper tube aligned in the A12 position.*

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- There are three Z Max positions that can be specified. One is for each type of plate available in the software. The Z Max setting will be for both rack 2 and for rack 3 if the same plate is used in both racks. Therefore, to set the Z Max find the Z parameter that just barely touches the bottom of the tube or well in the 4 corner positions for both racks. Select the Z parameter that will work for all 8 of these positions (left plate and right plate).

Select "OK" when all calibrations are done and the racks are set to save the new parameters.

Make sure to designate which type of rack is used in the Autosampler configuration screen when setting up experiments.

Note: If any of the 4000 or Autosampler calibrations are changed, then the microtiter plate calibration will need to be checked again before setting up an experiment using the microtiter plate.

Microtiter Sample Well locations

Designating the sample well locations is similar to the way the sample tubes are designated on other rack types. In the example shown in ***Figure 9A*** for rack 2, the sample set would read 201-212. The first digit (2) specifies rack 2, and the last two digits specify the well numbers. The well numbers move from right to left, then down, starting at the top right corner. For more information see Tech Note 209 *Software Shortcuts* (**TN209**) for specifying sample sets.

An alternate way of designating samples is to use the row and column designations normally used for microtiter plates. See the example shown in ***Figure 9B*** for rack 3. The sample set would read 3:A1-B4. The number before the colon (3) specifies the rack, and the numbers after the colon specifies the wells. The sample selection moves down, then right to left, starting at the top right corner.

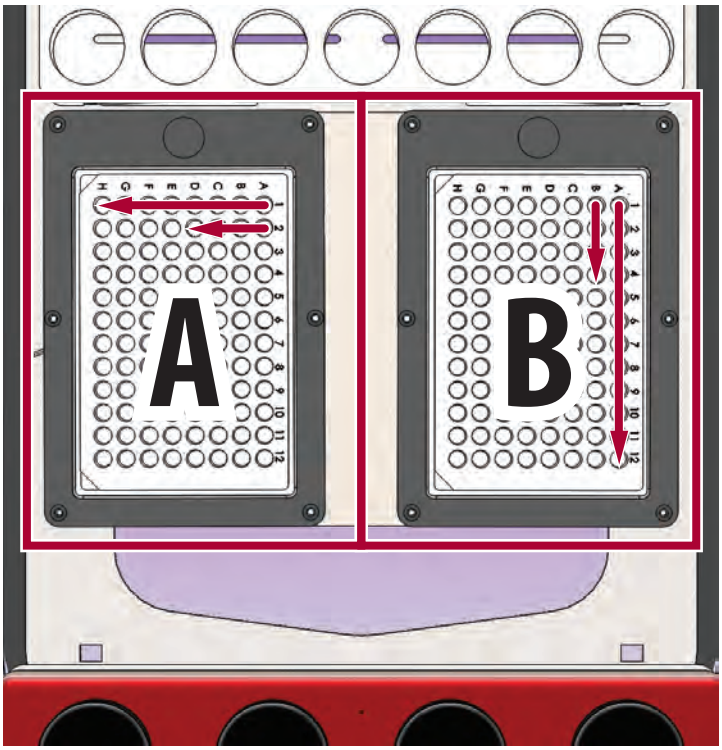

Figure 9A & B. *Designating samples.* ***A.*** *Standard way to designate samples.* ***B.*** *Alternate way to designate samples.*

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