

Sample name: **M12**
 Assay name: **UV-metric pKa**
 Assay ID: **17I-21004**
 Filename: **C:\Sirius_T3\Mehtap\20170920_exp05_M01-M14\17I-21004_M12_UV-metric pKa.t3r**

Experiment start time: **9/21/2017 1:51:22 AM**
 Analyst: **Dorothy Levorse**
 Instrument ID: **T311053**

Results

pKa 1 **5.27**
 pKa 2 **12.53**
 RMSD **0.010 0.004 0.003**
 Chi squared **0.0568**
 PCA calculated number of pKas **2**
 Average ionic strength **0.158 M**
 Average temperature **24.9°C**
 Analyte concentration range **70.0 µM to 63.3 µM**

Number of pKas source **Manual (2)**
 Wavelength clipping **230.0 nm to 450.0 nm**
 pH clipping **1.269 to 12.711**

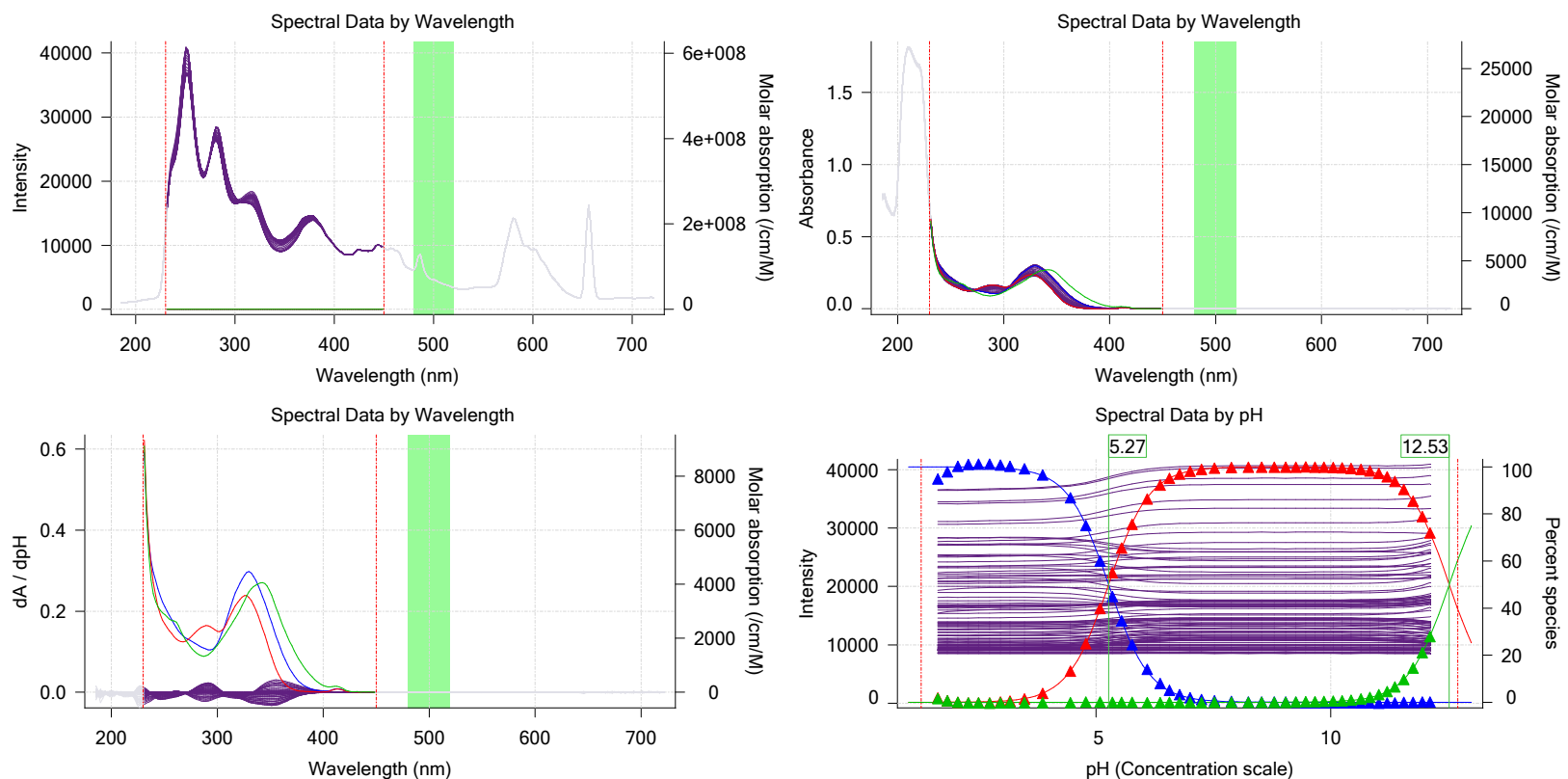
Warnings and errors

Errors **None**
 Warnings **PCA calculation disagrees with predicted number of pKas**

Assay Settings

| Setting | Value | Original Value | Date/Time changed | Imported from |
|-----------------------------|------------------|----------------|-------------------|---------------|
| Buffer in use | Yes | | | |
| Buffer type | Phosphate Buffer | | | |
| Assay Medium | | | | |
| Volume of buffer introduced | 0.025000 mL | | | |
| Add buffer manually | Manual | | | |

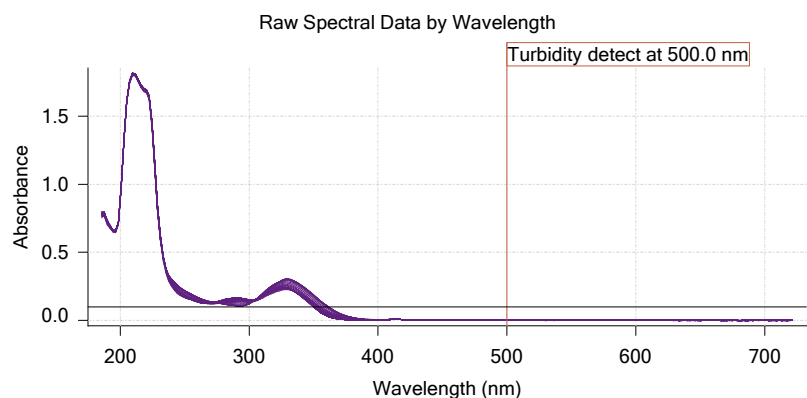
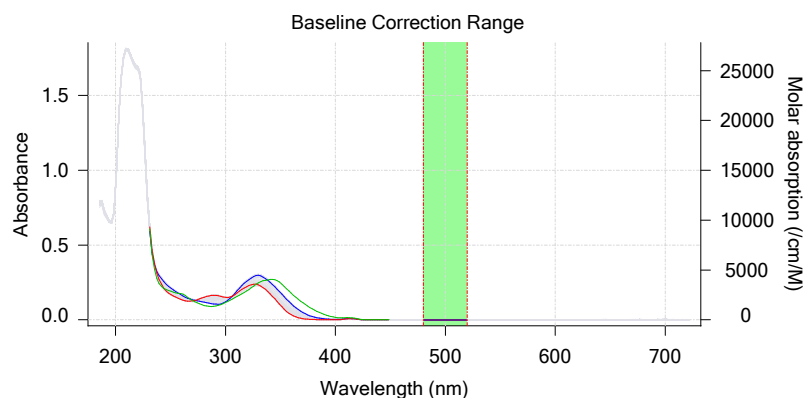
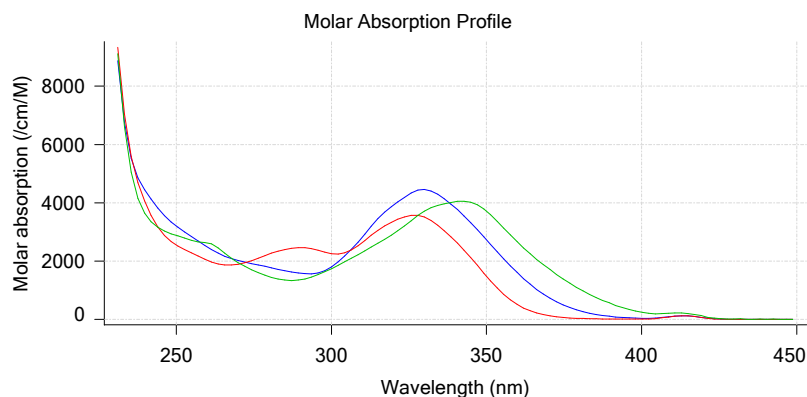
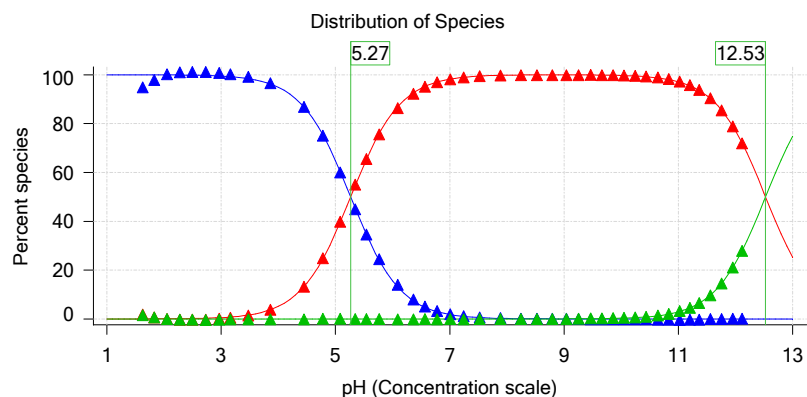
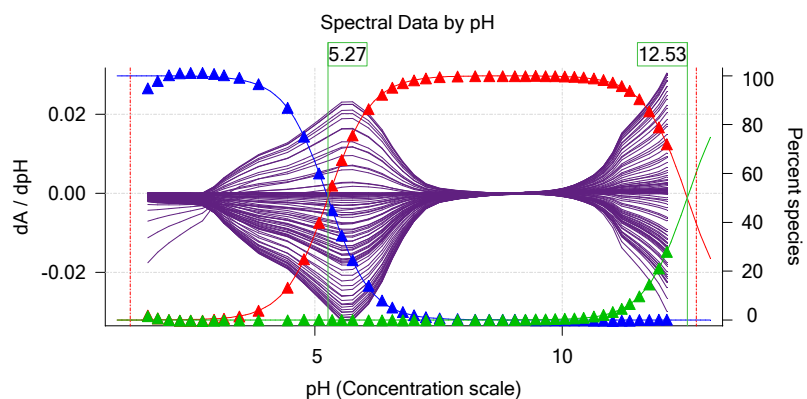
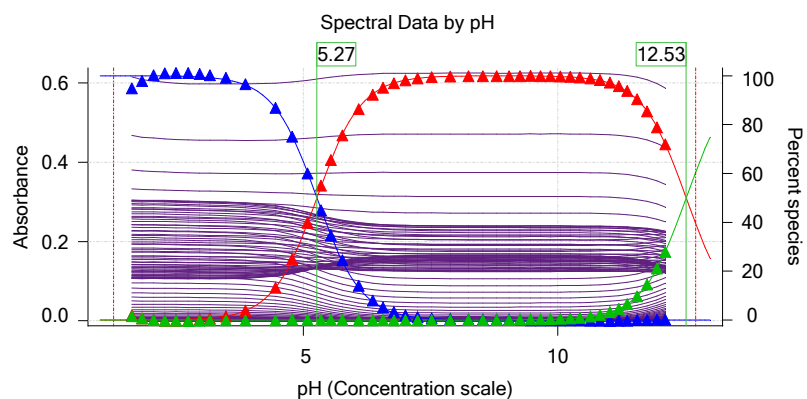
Graphs



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 Analyst: **Dorothy Leverse**
 Instrument ID: **T311053**

Graphs (continued)



Assay Model

Settings

| Sample name | M12 | 9/20/2017 2:59:05 PM | User entered value |
|-----------------------------------|------------|----------------------|--------------------|
| Sample by | Volume | | Default value |
| Sample volume | 0.0030 mL | 9/20/2017 2:59:05 PM | User entered value |
| Solvent | DMSO | | Default value |
| Sample concentration | 0.037300 M | 9/20/2017 2:59:05 PM | User entered value |
| Solubility | Unknown | | Default value |
| Molecular weight | 292.15 | 9/20/2017 2:59:15 PM | User entered value |
| Individual pKa ionic environments | No | | Default value |
| Number of pKas | 1 | 9/20/2017 2:59:05 PM | User entered value |
| Sample is a | Base | 9/20/2017 2:59:05 PM | User entered value |
| pKa 1 | 5.60 | 9/20/2017 2:59:05 PM | User entered value |
| logp (XH +) | -10.00 | | Default value |
| logP (neutral X) | -10.00 | 9/20/2017 2:59:05 PM | User entered value |
| Stoichiometry | 1.00000 | | Default value |

| | | | |
|--------------|---|------------------------|-----------------------------|
| Sample name: | M12 | Experiment start time: | 9/21/2017 1:51:22 AM |
| Assay name: | UV-metric pKa | Analyst: | Dorothy Levorse |
| Assay ID: | 17I-21004 | Instrument ID: | T311053 |
| Filename: | C:\Sirius_T3\Mehtap\20170920_exp05_M01-M14\17I-21004_M12_UV-metric pKa.t3r | | |

Assay Model (continued)

| Settings | Value | Date/Time changed | Imported from |
|-------------------------|----------|-------------------|-------------------------|
| Aprotic counterion name | Chloride | | From standards.xml file |
| Stoichiometry | 1.00 | | From standards.xml file |
| Charge per counterion | -1 | | From standards.xml file |

Assay Settings

| Setting | Value | Original Value | Date/Time changed | Imported from |
|-------------------------------------|--------------------|----------------|-------------------|---------------|
| General Settings | | | | |
| Analyst name | Dorothy Levorse | | | |
| Separate reference vial | Yes | | | |
| Standard Experiment Settings | | | | |
| Number of titrations | 1 | | | |
| Minimum pH | 1.800 | | | |
| Maximum pH | 12.200 | | | |
| pH step between points of | 0.200 | | | |
| Minimum titrant addition | 0.00002 mL | | | |
| Maximum titrant addition | 0.10000 mL | | | |
| Argon flow rate | 100% | | | |
| Start titration using | Cautious pH adjust | | | |
| Advanced General Settings | | | | |
| Detect turbidity using | Spectrometer | | | |
| Monitor at a wavelength of | 500.0 nm | | | |
| Absorbance threshold of | 0.100 | | | |
| Collect turbidity sensor data | No | | | |
| Stir after titrant addition for | 5 seconds | | | |
| For titrant addition, stir at | 15% | | | |
| Titrant Pre-Dose | | | | |
| Titrant pre-dose | None | | | |
| Assay Medium | | | | |
| Cosolvent in use | No | | | |
| ISA water volume | 1.50 mL | | | |
| Water added | Automatic | | | |
| After water addition, stir for | 5 seconds | | | |
| At a speed of | 15% | | | |
| Buffer in use | Yes | | | |
| Buffer type | Phosphate Buffer | | | |
| Volume of buffer introduced | 0.025000 mL | | | |
| Add buffer manually | Manual | | | |
| After medium addition, stir for | 5 seconds | | | |
| Sample Sonication | | | | |
| Sonicate | No | | | |
| Sample Dissolution | | | | |
| Perform a dissolution stage | No | | | |
| Carbonate purge | | | | |
| Perform a carbonate purge | No | | | |
| Temperature Control | | | | |
| Wait for temperature | Yes | | | |
| Required start temperature | 25.0°C | | | |
| Acceptable deviation | 0.5°C | | | |
| Time to wait | 60 seconds | | | |
| Stir speed of | 15% | | | |
| Titration 1 | | | | |
| Titrate from | Low to high pH | | | |
| Adjust to start pH | Yes | | | |
| After pH adjust stir for | 10 seconds | | | |
| Data Point Stability | | | | |
| Stir during data point collection | Yes | | | |
| For point collection, stir at | 15% | | | |

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Assay Settings (continued)

| Setting | Value | Original Value | Date/Time changed | Imported from |
|-------------------------------------|----------------|----------------|-------------------|---------------|
| Delay before data point collection | 0 seconds | | | |
| Number of points to average | 20 points | | | |
| Time interval between points | 0.50 seconds | | | |
| Required maximum standard deviation | 0.00500 dpH/dt | | | |
| Stability timeout after | 60 seconds | | | |
| Experiment cleanup | | | | |
| Adjust pH to cleanup | To start pH | | | |
| And then stir for | 60 seconds | | | |
| For cleaning, stir at | 20% | | | |
| Then add water volume | 0.25 mL | | | |
| And then stir for | 30 seconds | | | |

Calibration Settings

| Setting | Value | Date/Time changed | Imported from |
|---------------------------|--------|----------------------|--|
| Four-Plus alpha | 0.143 | 9/21/2017 1:51:22 AM | C:\Sirius_T3\17I-20017_Blank standardisation.t3r |
| Four-Plus S | 0.9975 | 9/21/2017 1:51:22 AM | C:\Sirius_T3\17I-20017_Blank standardisation.t3r |
| Four-Plus jH | 0.3 | 9/21/2017 1:51:22 AM | C:\Sirius_T3\17I-20017_Blank standardisation.t3r |
| Four-Plus jOH | -0.8 | 9/21/2017 1:51:22 AM | C:\Sirius_T3\17I-20017_Blank standardisation.t3r |
| Base concentration factor | 1.015 | 9/21/2017 1:51:22 AM | C:\Sirius_T3\KOH17I11.t3r |
| Acid concentration factor | 1.008 | 9/21/2017 1:51:22 AM | C:\Sirius_T3\17I-20017_Blank standardisation.t3r |

Instrument Settings

| Setting | Value | Batch Id | Install date |
|----------------------|----------------------------|-------------|------------------------|
| Instrument owner | Merck | | |
| Instrument ID | T311053 | | |
| Instrument type | T3 Simulator | | |
| Software version | 1.1.3.0 | | |
| Dispenser module | | T3DM1100253 | 3/31/2009 6:24:52 AM |
| Dispenser 0 | Water | | 3/31/2009 6:25:05 AM |
| Syringe volume | 2.5 mL | | |
| Firmware version | 1.2.1(r2) | | |
| Titrant | Water (0.15 M KCl) | 8-18-17 | 9/18/2017 9:13:04 AM |
| Dispenser 2 | Acid | | 3/31/2009 6:25:11 AM |
| Syringe volume | 0.5 mL | | |
| Firmware version | 1.2.1(r2) | | |
| Titrant | Acid (0.5 M HCl) | 166940 | 9/8/2017 9:21:27 AM |
| Dispenser 1 | Base | | 3/31/2009 6:25:21 AM |
| Syringe volume | 0.5 mL | | |
| Firmware version | 1.2.1(r2) | | |
| Titrant | Base (0.5 M KOH) | 01/06/17 | 9/8/2017 9:20:03 AM |
| Dispenser 5 | Cosolvent | | 3/31/2009 6:26:24 AM |
| Syringe volume | 2.5 mL | | |
| Firmware version | 1.2.1(r2) | | |
| Distribution valve 5 | Distribution Valve | | 3/31/2009 6:28:19 AM |
| Firmware version | 1.1.3 | | |
| Port A | Methanol (80%, 0.15 M KCl) | 8-15-17 | 9/20/2017 4:38:16 PM |
| Port B | Cyclohexane | | 9/19/2017 2:15:02 PM |
| Dispenser 3 | Buffer | | 8/3/2010 6:05:16 AM |
| Syringe volume | 0.5 mL | | |
| Firmware version | 1.2.1(r2) | | |
| Titrant | Phosphate Buffer | | 9/12/2017 12:32:29 PM |
| Dispenser 6 | Octanol | | 10/22/2010 11:52:43 AM |
| Syringe volume | 0.5 mL | | |
| Firmware version | 1.2.1(r2) | | |
| Titrant | Octanol | 9-14-17 | 9/14/2017 10:30:38 AM |
| Titration | | T3TM1100153 | 3/31/2009 6:24:17 AM |



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Instrument Settings (continued)

| Setting | Value | Batch Id | Install date |
|---|----------------------------|-------------|------------------------|
| Horizontal axis firmware version | 1.17 AI1DI2DO2 Stepper 2 | | |
| Vertical axis firmware version | 1.17 AI1DI2DO2 Stepper 2 | | |
| Chassis I/O firmware version | 1.11 AI1DI0DO4 Norgren I/O | | |
| Probe I/O firmware version | 1.1.1 | | |
| Electrode | T3 Electrode | T3E0769 | 8/15/2017 10:21:54 AM |
| E0 calibration | -8.78 mV | | 9/21/2017 1:51:46 AM |
| Filling solution | 3M KCl | KCL095 | 9/18/2017 9:17:15 AM |
| Liquids | | | |
| Wash 1 | 50% IPA:50% Water | | 9/20/2017 4:35:48 PM |
| Wash 2 | 0.5% Triton X-100 in H2O | | 9/20/2017 4:35:52 PM |
| Buffer position 1 | pH7 Wash | | 9/20/2017 4:35:55 PM |
| Buffer position 2 | pH 7 | | 9/20/2017 4:35:58 PM |
| Storage position | | | 9/20/2017 4:36:03 PM |
| Wash water | 3e+003 mL | 9-18-17 | 9/18/2017 8:54:32 AM |
| Waste | 7e+003 mL | | 9/18/2017 8:54:39 AM |
| Temperature controller | | | 8/5/2010 7:35:13 AM |
| Turbidity detector | | | 3/31/2009 6:24:45 AM |
| Spectrometer | | 072390 | 11/23/2010 12:22:28 PM |
| Dip probe | | 11086 | |
| Wavelength coefficient A0 | 185.563 | | |
| Wavelength coefficient A1 | 2.17439 | | |
| Wavelength coefficient A2 | -0.000285622 | | |
| Total lamp lit time | 172:20:49 | | 11/23/2010 12:22:28 PM |
| Calibrated on | 9/18/2017 9:35:14 AM | | |
| Integration time | 11 | | |
| Scans averaged | 10 | | |
| Autoloader | | T3AL1100237 | 11/10/2015 10:34:13 AM |
| Left-right axis firmware version | 1.17 AI1DI2DO2 Stepper 2 | | |
| Front-back axis firmware version | 1.17 AI1DI2DO2 Stepper 2 | | |
| Vertical axis firmware version | 1.17 AI1DI2DO2 Stepper 2 | | |
| Chassis I/O firmware version | 1.11 AI1DI0DO4 Norgren I/O | | |
| Configuration | | | |
| Alternate titration position | Titration position | | |
| Alternate reference position | Reference position | | |
| Maximum standard vial volume | 3.50 mL | | |
| Maximum alternate vial volume | 25.00 mL | | |
| Automatic action idle period | 5 minute(s) | | |
| Titrant tube volume | 1.3 mL | | |
| Syringe flush count | 3.50 | | |
| Flowing wash pump volume | 20.0 mL | | |
| Flowing wash stir duration | 5 s | | |
| Flowing wash stir speed | 30% | | |
| Solvent wash stir duration | 5 s | | |
| Solvent wash stir speed | 30% | | |
| Surfactant wash stir duration | 5 s | | |
| Surfactant wash stir speed | 30% | | |
| E0 calibration minimum number of points | 10 | | |
| E0 calibration maximum standard deviation | 0.01500 | | |
| E0 calibration timeout period | 60 s | | |
| E0 calibration stir duration | 5 s | | |
| E0 calibration preparation stir speed | 30% | | |
| E0 calibration buffer wash stir duration | 5 s | | |
| E0 calibration buffer wash stir speed | 30% | | |
| E0 calibration reading stir speed | 0% | | |
| Spectrometer calibration stir duration | 5 s | | |
| Spectrometer calibration stir speed | 30% | | |
| Spectrometer calibration wash pump volume | 20.0 mL | | |
| Spectrometer calibration wash stir duration | 5 s | | |



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Instrument Settings (continued)

| Setting | Value | Batch Id | Install date |
|--|-------|----------|--------------|
| Spectrometer calibration wash stir speed | 30% | | |
| Overhead dispense height | 10000 | | |

Refinement Settings

| Setting | Value | Default value |
|---------------------------------------|--------------|---------------|
| Turbidity detection method | Spectrometer | Spectrometer |
| Turbidity wavelength to assess | 500.0 nm | 500.0 nm |
| Turbidity maximum absorbance | 0.100 | 0.100 |
| Turbidity probe threshold | 50.00 | 50.00 |
| Exclude turbid points | Yes | Yes |
| Low intensity warning threshold | 100 | 100 |
| Minimum absorbance change threshold | 0.100 | 0.100 |
| Eigenvector autocorrelation threshold | 0.80 | 0.80 |
| Maximum RMSD severe warning | 0.250 | 0.250 |
| Maximum RMSD warning | 0.050 | 0.050 |

Tray Information

Title
Location E5