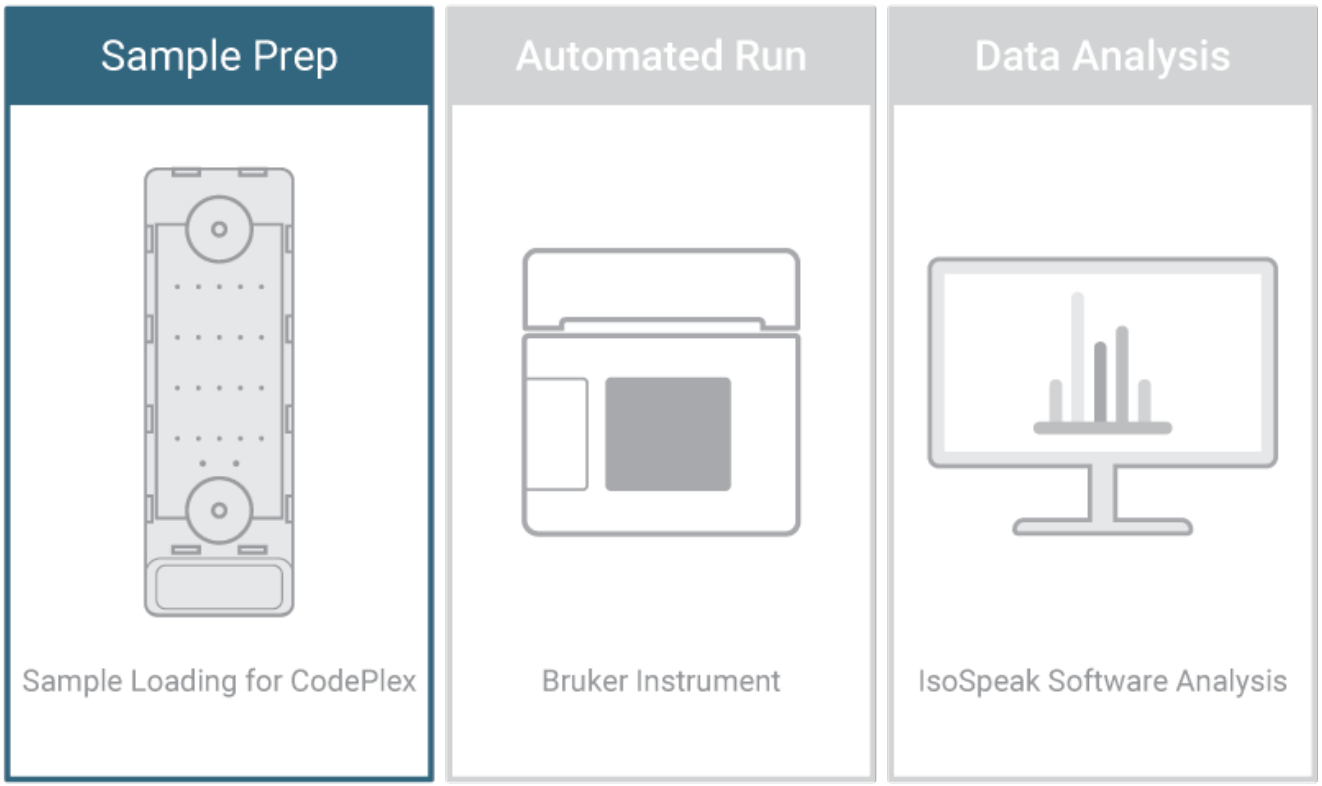
CodePlex Secretome

Ensure you achieve the maximum benefit from the Bruker systems and generate impactful data as quickly as possible



Contents

A. Overview

1. Overview of Protocol

Day 1: Thaw and load samples and background controls into CodePlex chip.

NOTE:

This protocol outlines the standard method for thawing and loading samples onto the CodePlex chip. See Appendix 2 for abbreviated loading instructions.

For first time users, it is highly recommended to first practice loading with a CodePlex Training Chip, which is provided for new users with their first order.

2. Safety Warnings

- Read MSDS documents of all materials prior to use.

- Laboratory workers should wear standard PPE, including disposable gloves, protective eyewear, and laboratory coats.

3. Required Reagents, Consumables, and Equipment

Table 1: Required Reagents and Consumables Provided by Bruker

Item	Catalog Number	Quantity	Comment
CodePlex Kit	Please see website (https://brukercellularanalysis.com/) for available kits or talk to Bruker's Customer Service team for details	One chip per 8 samples	Subcomponents stored at 4°C and -20°C

CodePlex Kit Components

IsoLight CodePlex Reagent Box (Store at 4°C)

- 15 mL Tube A
- 15 mL Tube B
- 1.5 mL Tubes A/B: Cocktail A in Micro-Tube (Green Cap) and Cocktail B in Micro-Tube (Red Cap)
- 50 mL Tubes containing Reagents 1, 2, 3, 4, 5, 6, 7, 8
- 1 Bag of Disposable Reagent Sippers

IsoSpark CodePlex Reagent Box (Store at 4°C)

- 15 mL Tube A
- 15 mL Tube B
- 1.5 mL Tubes A/B: Cocktail A in Micro-Tube (Green Cap) and Cocktail B in Micro-Tube (Red Cap)
- Cartridge containing Reagents 1, 2, 3, and 4

CodePlex Chip Set (Store at -20°C)

- Boxes of CodePlex Chips
  - IsoSpark: 1, 2 or 4 chips
  - IsoLight: 1, 2, 4, 6, or 8 chips

CodePlex Required Accessories (Store at Room Temperature)

- Cover Tape (One per chip)
- Cover Tape Applicator (One per kit)
- P10 Filtered Pipette Tips (One rack for 1, 2 and 4 chips; two racks for 6 and 8 chips)*
- CodePlex Calibration Chip
- CodePlex Training Chip (new users only)

*NOTE: P10 filtered pipette tips provided as part of the CodePlex kit are not compatible with Rainin LTS pipettes.

Table 2: Required Consumables Not Supplied by Bruker

Consumable	Type	Source	Catalog Number
Fisherbrand Disposable PES Filter Units (0.20 µm)	500 mL	Fisher Scientific	FB12566504

Table 3: Required Reagents Not Supplied by Bruker

Reagent	Stock Concentration	Source	Catalog Number
RPMI	1x	Fisher	MT10040CV
Penicillin-Streptomycin-Neomycin Solution Stabilized	100x	Sigma	P4083-100mL
Glutamax	100x	Thermo	35050061
FBS	1x	Sigma	F2442-6X500mL
Bovine Serum Albumin (BSA), lyophilized powder	N/A	Sigma-Aldrich	A9647-10G
Bovine Serum Albumin (BSA), Lyophilized powder, Globulin Free, Low Endotoxin	N/A	Sigma-Aldrich	A2934-25G
Phosphate Buffered Saline (1XPBS) without Calcium or Magnesium	1x	Gibco	10010072

Table 4: Required Equipment

Equipment	Source	Catalog Number/Requirements
IsoLight, IsoSpark, or IsoSpark Duo Instrument	Bruker	ISOLIGHT-1000-1, ISOSPARK-1000-1, or ISOSPARK-1001-1

Table 5: General Equipment

Equipment	Requirements
Pipette	P10, P100, P1000

B. Before Getting Started

1. Important Precautions

Read MSDS documents of all materials prior to use.

Working with Biohazardous Reagents

Please refer to your institute's guidelines and obtain proper training to handle potentially biohazardous samples. It is also strongly recommended that any lab personnel handling human samples should be vaccinated against HBV if the individual does not have sufficient HBV antibody titer.

Additional precautions need to be taken when working with samples that potentially contain an EID agent:

1. Laboratory workers should wear standard PPE, including disposable gloves, protective eyewear, and laboratory coats.
2. Any procedure or process that cannot be conducted in the designated EID BSC should be performed while wearing gloves, gown, goggles and a fit tested N-95 mask.
3. Work surfaces should be decontaminated on completion of work with appropriate disinfectants. This includes any surface that potentially comes in contact with the specimen (centrifuge, microscope, etc.).
4. All liquid waste produced in the processes must be treated to a final concentration of 10% bleach prior to disposal.

2. Reagents to Be Prepared Before Starting

Table 6: Complete RPMI Recipe

- CRITICAL: Complete RPMI media has been validated for use by Bruker. Using alternative media may result in failed runs. Please contact your Field Application Scientist for additional information.

Ingredient	Stock Concentration	Final Concentration	Amount for 500 mL	Vendor/Catalog
Penicillin-Streptomycin- Neomycin Solution Stabilized	100x	1x	5 mL	Sigma P4083-100mL
Glutamax	100x	1x	5 mL	Thermo/35050061
FBS	100%	10%	50 mL	Sigma/F2442-6X500 mL
RPMI	1x	1x	440 mL	Fisher/MT10040CV

Note | Sterile-filter through 0.20 µm filter before use. Store complete RPMI Media at 4°C.

Table 7: 2% BSA Recipe

Ingredient	Stock Concentration	Final Concentration	Amount for 100 mL	Vendor/Catalog
Bovine Serum Albumin (BSA), Lyophilized powder or Bovine Serum Albumin (BSA), Lyophilized powder, Globulin Free, Low Endotoxin	N/A	2%	2g	Sigma-Aldrich/A9647-10G or Sigma-Aldrich/ A2934-25G
Phosphate Buffered Saline (1X PBS) without Calcium or Magnesium	1X	1X	99mL initially*	Gibco/10010072

*Rotate solution until BSA powder is dissolved and then bring final volume up to 100mL with 1X PBS.

3. Guidelines for Sample Stability

Bruker recommends the following general guidelines for storing your sample. This is not intended to be an all-inclusive listing. Please refer to your institute's guidelines for long term sample storage if applicable.

1. Avoid repeated freeze-thaw cycles of samples as this may degrade, partially or fully, the sample quality.
    a. If a sample needs to be run multiple times, aliquot smaller volumes into single-use low protein binding tubes and thaw as needed.
2. For cell supernatant, store samples in a low protein binding tube.
3. Cell supernatant continuously stored at -80°C should be stable for at least 1 year.
    a. If possible, arrange to run samples as soon as possible to minimize duration-based degradation.
    b. Literature reports suggest stability for up to 2 years in most cases. However, Bruker has not independently verified this information, and in general urges caution when running extremely old samples.
4. When collecting cell supernatants, be sure to save an aliquot of the same media batch used during supernatant generation for use as the Background Control.

C. Protocol

Day 1: Thawing and Loading

Materials Required

CodePlex Kit Components
Samples: Compatible Sample Types Listed in Table 8
Background Control: Sample Type Specific, Table 9

Table 8: Products and Sample Compatibility

CodePlex Secretome Product	Cell Culture Supernatant	Plasma, Serum	Cerebrospinal Fluid (CSF)	Lung Lavage / Tracheal Wash	Urine*
Adaptive Immune - Human	✓	✓	✓	✓	✓
Cytokine Storm - Human	✓	✓	✓	✓	✓
Stem Cell Signaling - Human	✓	✓			
Cancer Signaling - Human	✓	✓			
Innate Immune - Human	✓	✓			
Adaptive Immune - Mouse	✓	✓			
Inflammation - Mouse	✓	✓			
Innate Immune - Mouse	✓	✓			
Adaptive Immune - NHP	✓	✓			

*Guidelines for Urine Samples for Analysis on CodePlex Secretome

Urine samples need to be processed prior to loading on the CodePlex Secretome chip utilizing the following method.

1. After sample collection, centrifuge sample for 10 minutes at 2000 rcf at room temperature.
2. Remove supernatant from particulate pellet formed by centrifugation.
3. Sample may now be loaded onto CodePlex chips. It is recommended that the samples be run undiluted to maintain intensity of each cytokine signature. Samples may also be aliquoted and stored at -80°C for later analysis.
4. Urine samples may be normalized using a creatinine assay per industry standard. This normalization is conducted separately from the CodePlex Secretome assay.

Table 9: Sample Type and Background Control / Sample Diluent

Sample Type	Background Control / Sample Diluent (if necessary)
Cell Supernatant	Complete RPMI, from same media batch as supernatants (Table 6 for Recipe)
Plasma, Serum	2% BSA in PBS* Solution made and used same day
Cerebrospinal Fluid (CSF)	
Lung Lavage / Tracheal Wash	
Urine	2% BSA (globulin free, low endotoxin) in PBS* Solution made and used same day

Note: Use only Background Controls in Table 9. The same Background Control must be loaded in all background wells on a chip. You can load different samples on a chip if they use the same Background Control.

*2% globulin free, low endotoxin BSA in PBS has been validated for use as a background with urine samples. Using an alternative BSA may result in failed runs. 2% globulin free, low endotoxin BSA in PBS may also be substituted for the standard 2% BSA in PBS.

Methods

- 1. Remove vacuum sealed bag containing CodePlex chips from -20ºC.
  CRITICAL: Chips must stay sealed until loading.

2. Place CodePlex chips on a bench to thaw in the vacuum sealed bag at ambient temperature 60 to 75 minutes prior to opening the vacuum bag.

- 3. Allow frozen samples to completely thaw at room temperature. TIP: Mix well by pipetting up and down prior to loading. Use a larger volume pipette (e.g., 100-1000 µL) to mix, depending on volume of sample. P10 pipette used to dispense sample into the chip will not provide adequate mixing for volumes greater than ~25 µL.

4. Optimally, while chips and samples thaw, prepare CodePlex liquid reagents and setup in the Bruker instrument. Refer to your instrument's system guide for detailed instructions.

- 5. Once thawed, remove CodePlex chips from vacuum sealed bag and place on a flat surface.
  CRITICAL: Keep protective blue film on bottom of chip.

- CRITICAL: Each well of the CodePlex chip must be loaded with sample or background control in numerical order and each well of a row must be filled before loading the wells of the next row. Wells 5, 6, 15, and 16 are labeled "B" and are designated for loading background controls, all other wells may be loaded with sample or background. Use only the Background Controls indicated in Table 9 and load all four Background wells with the same control fluid. All samples are loaded in duplicate wells and both wells are required to run the assay correctly.

- 6. Using a P10 pipette, load 5.5 µL of Sample "a" into CodePlex well 1, firmly inserting the pipette tip into the well to ensure the pipette tip creates a seal around the well opening. Discard pipette tip. CRITICAL: Only dispense the sample to the first stop of the pipette to prevent bubbles from forming. DO NOT release the

plunger. With the plunger still held at the first stop, wait for 2 seconds for the sample to load, then slowly remove the tip from the well to avoid disturbing the sample.

NOTE: It is important to only use the P10 pipette tips supplied with the CodePlex kit as only certain pipette tips have been validated for use. Failure to do so can result in failure to create a seal between the pipette tip and the well opening. P10 pipette tips provided as part of the CodePlex kit are not compatible with Rainin LTS pipettes.

- 7. Repeat step 6 for duplicate loading of Sample "a" into CodePlex well 2.
  CRITICAL: Use a new pipette tip for each well to avoid introducing air bubbles into the sample.

Figure 1. Loading Sample into CodePlex Well

8. Load 5.5 µL of Sample "b" into CodePlex wells 3 and 4, as described in the previous steps.
9. Load 5.5 µL of the background control into well 5.

   NOTE: Wells 5, 6, 15, and 16 of the CodePlex chip are designated for loading background control and must not be loaded with sample.

10. After loading wells 1 through 5, invert the CodePlex chip and inspect sample fill length through the glass slide on the bottom of the chip. If any samples are filled less than 75% of the length between the well inlet and first sample divider of the next row (Figure 2), lightly tap chip parallel to benchtop (slide side down) to promote sufficient sample filling. Inspect sample fill in between tapping and stop once each sample has loaded at least 75% of the well length. Alternatively, chip may be lightly tapped perpendicular to benchtop (barcode side down) to promote filling.

- CRITICAL: Tap CodePlex chip lightly. Excessive force can cause sample contamination into the adjacent wells.

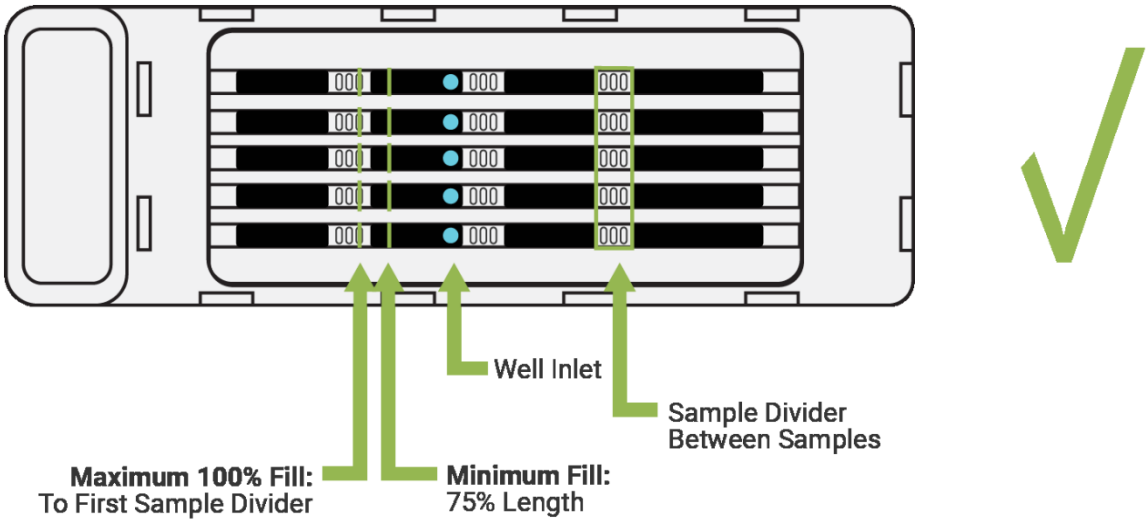


Figure 2. Properly Filled CodePlex Chip

11. Load 5.5 µL of background control into well 6.

- 12. Load remaining samples in duplicate into the remaining wells in order from well 7 to well 20, loading background controls into wells 15 and 16. CRITICAL: DO NOT load out of order. Loading out of order may result in sample cross-contamination.

- CRITICAL: If you have less than 8 samples, fill remaining wells with background control. All wells must be filled. You must load all 4 background wells. You must load in order.

- TIP: Invert chip and inspect fill volume through the glass slide after each row of 5 wells is loaded to ensure each sample has filled at least 75% of the well length before loading the next row.

- TIP: Refer to Sample Loading Template (Appendix 1).

13. After loading all samples and background controls in duplicate, gently invert chip to inspect sample loading through glass slide. As shown in Figure 2, liquid should cover at least 75% of the length of the sample chamber.

- TIP: In the unlikely event that an individual sample well has not loaded adequately, insert, by hand, a clean P10 pipette tip into the underfilled well inlet. Invert the chip while maintaining a gentle hold of the inserted pipette tip. Using gloved fingertip, lightly apply pressure on the exposed end of the tip, while observing sample through glass slide, to promote filling until minimum fill length achieved.

- CRITICAL: There should be a visible sample divider between each sample in each row. In the event of sample loading errors and/or contamination between adjacent samples, as shown in Figure 3, affected wells should be noted on sample loading template and excluded from IsoSpeak analysis.

Key: ● TIP, ● CRITICAL, ● OPTIONAL

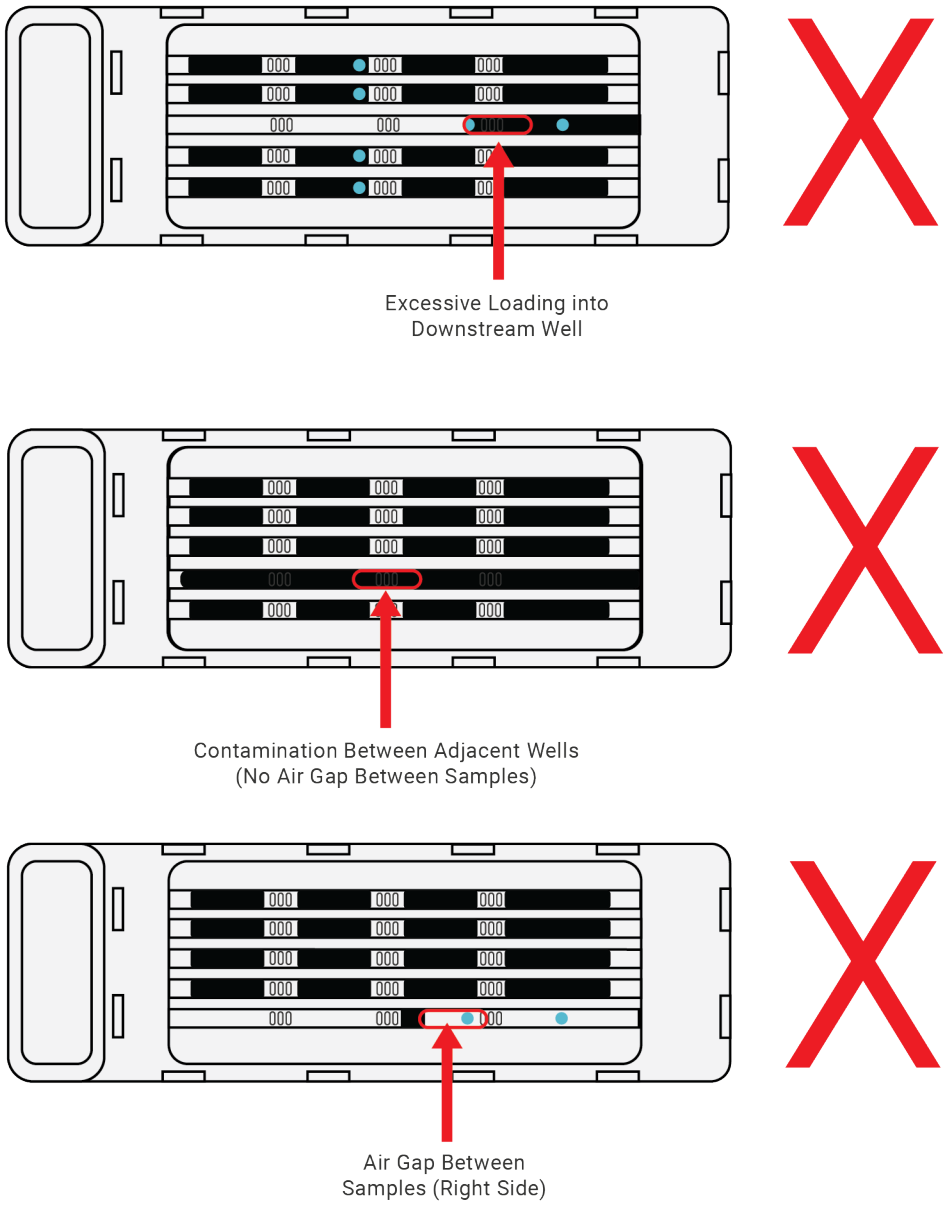


Figure 3. Examples of CodePlex Loading Errors

14. Once the CodePlex chip has been inspected for proper sample loading, apply the Cover Tape:

    a. Peel off the clear liner of the Cover Tape completely, exposing the adhesive side of the tape.

    b. Carefully align the Cover Tape to the top of the CodePlex chip, using the white rubber seals and outlined engravings on the chip as guides.

    c. Place the Cover Tape down and use a finger to apply even pressure to smooth and seal the tape across the entire surface of the CodePlex chip.

    d. Using the Cover Tape Applicator provided in the CodePlex Kit, apply moderate pressure across the Cover Tape to fully seal it to the chip. Slide the flat blade of applicator back and forth several times

over each portion of the tape, first lengthwise (Figure 4, Top and Center) and then widthwise (Figure 4, Bottom). TIP: Slide the blade until it touches the rubber seals on each end. Slight indents can be seen over the well inlets when sufficient sealing pressure is applied.

- CRITICAL: Failure to properly seal the CodePlex chip with Cover Tape may result in sample leakage, loss of data, and instrument damage. DO NOT touch the center hole of the white rubber seals on either end of chip, as this may cause cross contamination in adjacent samples.

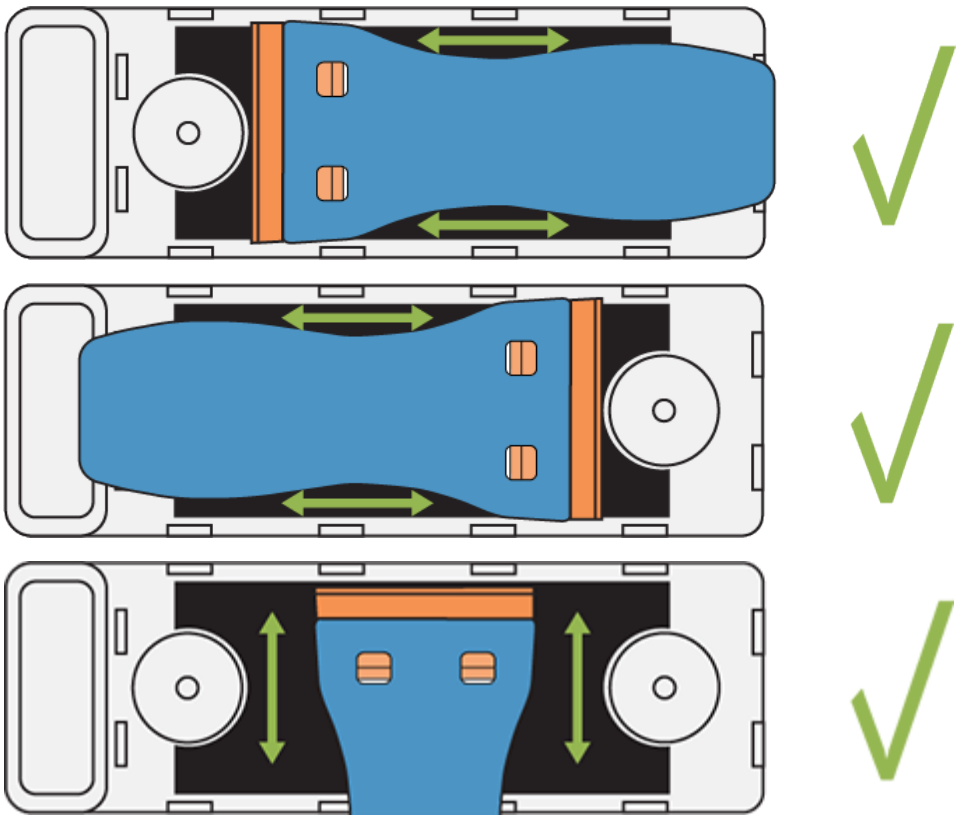


Figure 4. Use of Cover Tape Applicator on CodePlex Chip

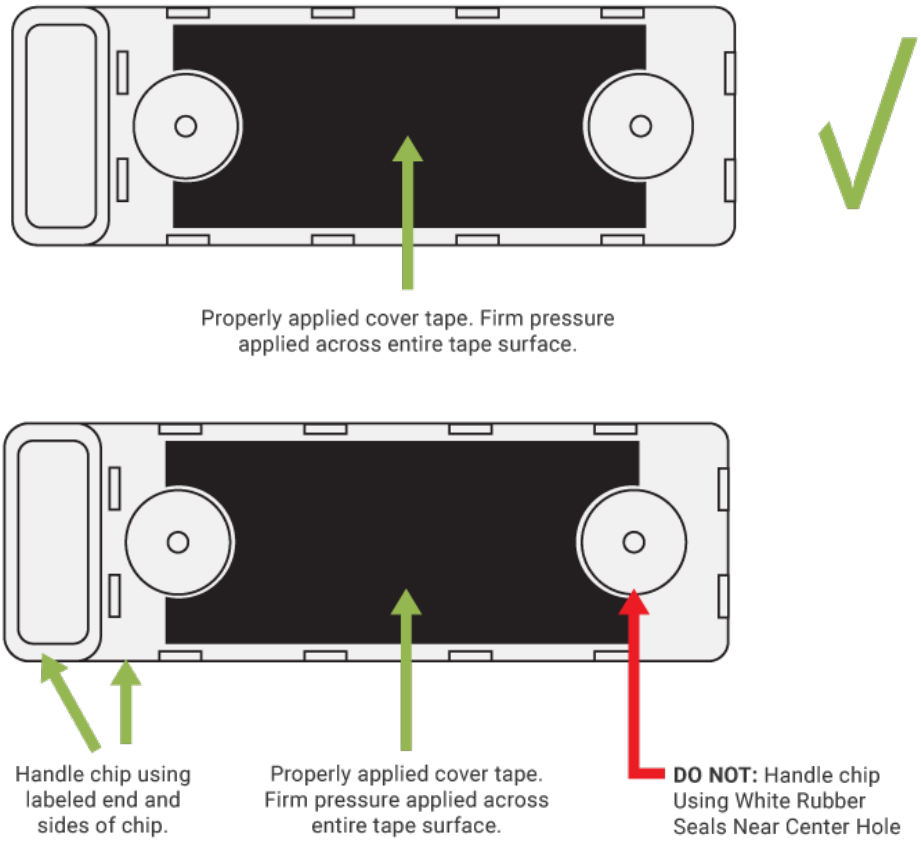


Figure 5. Properly sealed CodePlex chip

15. Once the CodePlex chip has been loaded and Cover Tape has been applied, perform a final brief inspection of sample fill length and sample dividers between samples through the glass slide.

Loading Chips in Instrument

16. Select CodePlex Secretome from Bruker instrument's primary screen. Load CodePlex Calibration Chip provided with the CodePlex Kit into tray position 4 for IsoLight or tray position 2 for IsoSpark when prompted, with small screw facing the instrument and "CAL" label facing up as shown in Figure 6. The Calibration Chip contains standard curve data and enables the instrument's system and IsoSpeak software to display concentrations in pg/mL.

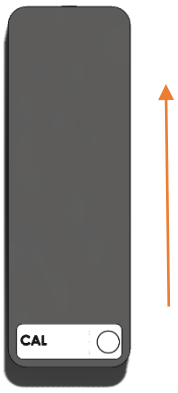


Figure 6. Proper Orientation for Loading Calibration Chip into Instrument

17. Continue following on screen prompts, and after removing Calibration Chip, proceed to load CodePlex Secretome chips into instrument chip tray according to your instrument's system guide.

- 18. Remove the blue protective tape from the bottom surface of the chip. Immediately load CodePlex chip into the instrument with the white rubber seals facing up and with the small screw facing the instrument. Chip should securely mate with the magnet in the chip tray. Continue loading all chips into tray.
CRITICAL: Handle CodePlex chips with care. Hold CodePlex chips by sides or barcode tab. DO NOT touch slide. DO NOT touch or apply pressure to the white rubber seals (inlet and outlet). DO NOT stack chips.

19. Verify that all CodePlex liquid reagents are properly setup in the instrument, prepared previously during chip thaw, and that the waste bottle is at least half empty (refer to your instrument's system guide for details).

20. Press "Close Chip Tray" and the instrument will start scanning the chip barcodes.
The instrument will also verify that the Cover Tape has been properly applied. If the cover tape is not detected, the Software will display an error and allow the user to open the tray to fix tape application.

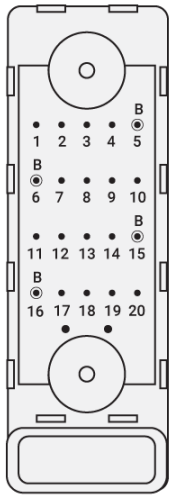
21. Press "Start Assay" after all chips are scanned.
Refer to your instrument's system guide for additional details and tips as necessary.

Appendix 1. CodePlex Sample Loading Template

CodePlex Chip Serial ID:				
Well 1	Well 2	Well 3	Well 4	Well 5
Sample a	Sample a	Sample b	Sample b	Background
Well 6	Well 7	Well 8	Well 9	Well 10
Background	Sample c	Sample c	Sample d	Sample d
Well 11	Well 12	Well 13	Well 14	Well 15
Sample e	Sample e	Sample f	Sample f	Background
Well 16	Well 17	Well 18	Well 19	Well 20
Background	Sample g	Sample g	Sample h	Sample h

Key: ● TIP, ● CRITICAL, ● OPTIONAL

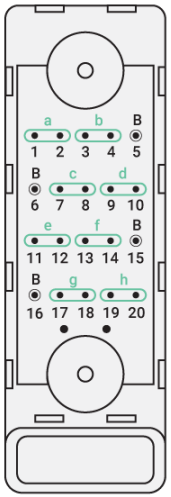
Appendix 2. CODEPLEX: STEPS TO LOAD YOUR CHIP



1 OVERVIEW: LAYOUT

The Codeplex chip consists of 20 wells and must be loaded in consecutive order from 1 to 20.

WARNING: Loading out of order will result in chip malfunction.

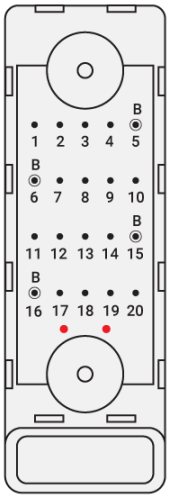


2 OVERVIEW: SAMPLE WELLS

Each sample is loaded into two wells (indicated in green):

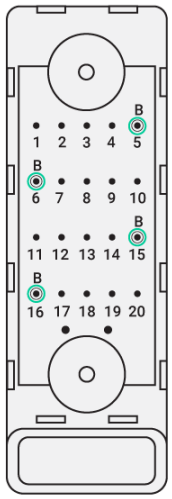
Sample Load Layout:
Sample a: wells 1 & 2
Sample b: wells 3 & 4
Sample c: wells 7 & 8
Sample d: wells 9 & 10
Sample e: wells 11 & 12
Sample f: wells 13 & 14
Sample g: wells 17 & 18
Sample h: wells 19 & 20

WARNING: Do not pipette sample out of order or in the incorrect well pairing. This will result in chip malfunction.



VENT PORTS

WARNING: Do not pipette into the unlabeled vent holes (indicated in red). This will result in chip malfunction.

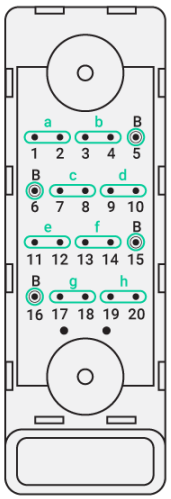


3 OVERVIEW: BACKGROUND WELLS

Each Background is loaded into the wells marked by a "B" (indicated in green):

Background: well 5
Background: well 6
Background: well 15
Background: well 16

WARNING: Do not load all of the Backgrounds at the same time. This will lead to chip malfunction. Follow loading instructions in step 4.

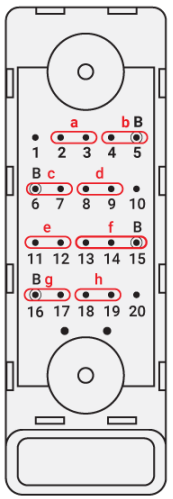


4 INSTRUCTIONS: HOW TO LOAD

Each sample and background must be loaded in consecutive order.

Sample a: wells 1 & 2
↓
Sample b: wells 3 & 4
↓
Background: well 5
↓
Background: well 6
↓
Sample c: wells 7 & 8
↓
Sample d: wells 9 & 10
↓
Continue sequence

WARNING: Loading out of order will result in chip malfunction.



SAMPLE LOADING ERROR

WARNING: Do not pipette sample out of order or in the incorrect well pairing (indicated in red). This will result in chip malfunction.