



Operator's Manual: Charm® SL-KIWI Beta-lactam Test for Raw Commingled Cow Milk

Kit Information

Introduction

The Charm SL-KIWI Beta-lactam Test is an immunoreceptor assay utilizing ROSA® (Rapid One Step Assay) lateral flow technology. Beta-lactams interact with colored beads in the lateral flow test strip, and the color intensity in the test and control zones is read visually or measured by the ROSA Reader. The Charm SL-KIWI Beta-lactam Test detects beta-lactam drugs at or below the New Zealand Maximum Residue Limit (Tables 1 and 2). The test is designed for use by dairy, intake, laboratory, field, and regulatory personnel.

Kit Contents and Materials Needed

Supplied with Kits	Disposables	Equipment
<ul style="list-style-type: none">SL-KIWIP Beta-lactam Test StripsPenicillin G Positive Tablets (supplied with 100K only)Operator's Manual	<ul style="list-style-type: none">Pipet Tips orROSA-Pipets	<ul style="list-style-type: none">ROSA Incubator (56°C) and ROSA Reader (optional) orCharm EZ (optional)Printer (optional)300 µl Pipet (optional)

Charm ROSA equipment available only from Charm Sciences, Inc.
Positive control may be purchased separately as needed [LF-PG-1-TBL].

Sensitivity and Selectivity

Beta-lactam-free raw milk yields negative results at least 99% of the time.
Sensitivity presented in Tables 1 and 2.

Storage

Store SL-KIWIP Beta-lactam test strips and Penicillin G Positive Tablets refrigerated (defined as 0 to 7°C or 0 to 4.5°C for US certified labs). See **Reagents and Storage** for details.

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© 2022 Charm Sciences, Inc. ROSA, Charm, Charm EZ and EZ Compatible names are trademarks of Charm Sciences, Inc. See www.charm.com/en/us-patents for a list of U.S. issued patents and pending, published U.S. and PCT applications.



Table 1. Sensitivity – Detection Levels and Concentration-Response in Cow Milk at 0 to 7°C – Displayed as Percent Positive (of n=30 tests).

Concentration (µg/kg)	Amoxicillin	Ampicillin	Ceftiofur [§]	Cephapirin	Penicillin G
1					0
2	3	3			13
3					73
4	70	13		0	100
5			0		100
6	100	83			
8	100	100		50	
10	100	97	0		
12				97	
16				100	
20			0	100	
40			0		
60			23		
80			100		
100			100		
New Zealand MRL (µg/kg)	4*	4*	100	10	4*
90% Positive with 95% Confidence ^{×§} (µg/kg)	5.6	8.5	77	13.7	3.6

§ The test sensitivity for ceftiofur was evaluated using milk containing ceftiofur and desfuroylceftiofur (DFC) related metabolites from treated animals. The ceftiofur tolerance is based on measuring the sum of ceftiofur and desfuroylceftiofur related metabolites in milk as desfuroylceftiofur.

× The 90% sensitivity with 95% confidence detection level is calculated based on U.S. FDA-CVM statistical analysis of independent lab blind study results. Results with the exception of ceftiofur are based on fortified raw milk samples.

* EU MRL by default (µg/kg).

Table 2. Sensitivity – Detection Range in Cow Milk at 0 to 7°C.

Beta-lactam Drug	Detection Range [†] (µg/kg)	New Zealand MRL (µg/kg)	Beta-lactam Drug	Detection Range [†] (µg/kg)	New Zealand MRL (µg/kg)
Cefacetrile	20 to 40	125*	Cefquinome	20 to 40	30
Cefalexin	30 to 70	100*	Cefuroxime	10 to 20	100*
Cefalonium	10 to 20	20*	Cloxacillin	30 to 70	30*
Cefazolin	30 to 50	50*	Dicloxacillin	30 to 70	30*
Cefoperazone	10 to 20	50*	Oxacillin	30 to 70	30*

† Positive at least 95% of the time.

* EU MRL by default (µg/kg).

Interferences and Cross Reactivity

There are no known interferences from drugs at 100 ppb in the following animal drug families: aminoglycosides, amphenicols, fluoroquinolones, macrolides/lincosamides, nitrofurans, tetracyclines, sulfa drugs/sulfonamides, or chlorothiazide, dexamethasone, dipyrone, flunixin, furosemide, ivermectin, novobiocin, oxytocin, PABA, phenylbutazone, trichlormethiazide, and thiabendazole. Other beta-lactam drugs are detected; cefadroxil at 30 to 70, nafcillin at 75 to 125, and ticarcillin at 75 to 125 µg/kg. There are no interferences from somatic cells at 10⁶ SCC/ml or bacteria at 3 x 10⁵ CFU/ml.

Training

- Equipment setup and use can be self-taught from the manual.
- Proficiency samples are available for validation.
- For questions contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.

Reagents and Storage

Test kits are not required to be shipped refrigerated.

Test Strips

- Store test strips refrigerated in tightly closed supplied container.
- To open test strip container, remove and save plastic lid with foil lined foam insert to reseal container. Lift foil tab and peel foil seal off container. Discard foil seal.
- In high humidity, limit condensation by opening container after it has warmed to room temperature (20 to 30 minutes from the time the container is removed from refrigerator).
- Inspect desiccant indicator in test strip container. Beads inside desiccant packets should be blue. Do not use test strips if blue beads have turned purple or pink.
- Remove from container the number of test strips to be used in one day; use supplied plastic lid to immediately reseal container tightly and return to refrigerated storage.
- Keep removed test strips at room temperature during daily use for up to 12 hours. Unused test strips should be discarded.

Negative Control

- Use beta-lactam negative raw, commingled milk as a Negative Control for performance monitoring and to reconstitute Positive Tablet for use as the Positive Control.
- Qualified Negative Control should yield a strong visual negative (**T** line is darker than **C** line). See **Visual Interpretation**.
- Store Negative Control refrigerated for up to 72 hours.
- See **Retest of Initial Positive** for Negative Control performance specifications.
- Use 300 µl of Negative Control in **Procedure**.

Positive Control

- Use Penicillin G Positive Tablet to make a 5.0 ± 0.5 ppb ($\mu\text{g}/\text{kg}$) penicillin G Positive Control for performance monitoring.
- Store Positive Tablets refrigerated in the closed zip-lock moisture resistant foil bag provided.
- Reconstitute one Positive Tablet with 5.0 ml of Negative Control (see above). Shake well. Allow to stand refrigerated or on ice for 5 minutes. Mix before use.
- Store reconstituted Positive Control refrigerated for up to 48 hours.
- See **Retest of Initial Positive** for Positive Control performance specifications.
- Use 300 µl of Positive Control in **Procedure**.

Long-Term Sample and Control Storage




- Mix sample, aliquot 0.5 ml portions (or more) into clean vials, label, and freeze.
- Freeze Controls within 6 hours of preparation.
- Freeze aliquots at -15°C or below for up to 2 months.
- Thaw frozen aliquots slowly (overnight in refrigerator or with cool water) and shake well. Store thawed sample or Controls refrigerated and use within 24 hours of thawing. Noticeable protein precipitation indicates an unsuitable sample.
- Discard any unused thawed sample. **Do not refreeze.**

Daily Performance Check

- Daily and before retesting “initial positive” samples, check ROSA Reader or Charm EZ performance using the supplied Calibration Strips. With ROSA Reader, enter performance mode in ROSA Reader by sequentially pressing ESC, 5, ENTER. With Charm EZ, go to menu and follow on-screen instructions. Calibration Strips must test within range.
- Test one Negative Control and one Positive Control prior to testing actual samples to verify performance of test strips and equipment. See **Retest of Initial Positive** for performance specifications for Controls.
- If Calibration Strips or Controls do not perform in specified ranges, discontinue use and contact Charm Sciences for assistance.

Precautions

- High fat samples (greater than 6.5%) may cause invalid results. Do not read invalid test strips in the ROSA Reader or Charm EZ.
- Debris on test strips may alter the ROSA Reader or Charm EZ optics. Keep equipment clean and wipe dust and milk off test strips before inserting in ROSA Reader or Charm EZ.
- ROSA Incubator or Charm EZ must be clean and level. ROSA Incubator or Charm EZ temperature must be $56 \pm 1^\circ\text{C}$. The temperature indicator should match ROSA Incubator temperature. A daily thermometer check is recommended. Keep ROSA Incubator lid lowered, but not latched, unless performing procedure.
- ROSA Incubators may take more than 10 minutes to reach proper temperature, depending on ambient temperature.
- The Charm EZ may take up to 3 minutes to reach proper temperature, depending on ambient temperature.
- Turning on the Charm EZ without the SD Card or the Slide Mechanism properly inserted will result in an error message.

<p>Charm EZ </p> <p>This strip is not Charm EZ compatible. Remove strip and insert correct type.</p>	<ul style="list-style-type: none"> • The Charm EZ is not compatible with all Charm test strips. • Older, non-compatible test strips will result in an error message when inserted into the Charm EZ. • For a complete list of Charm EZ compatible tests contact your local representative or Charm Sciences at +1.978.687.9200 or support@charm.com.
<p>Charm EZ </p> <p>Test has previously been run (lines detected) or reader lens is dirty. Check lens and run another strip.</p>	<ul style="list-style-type: none"> • Debris on test strips may alter the Charm EZ optics. • Keep equipment clean and wipe dust and milk off test strips before inserting in Charm EZ. • At the beginning of each assay run the Charm EZ checks to make sure the strip inserted has not been previously run and the optics lens is clean.
<p>Charm EZ </p> <p>ERROR. Door was opened during assay. Remove used strip and discard.</p>	<ul style="list-style-type: none"> • Do not open Charm EZ door while an assay is in progress. • Opening the door will invalidate the results and this will result in an error message. • Remove the test strip and restart the assay.

Charm
EZ



Flow error detected.
Run another test and
check volume dispensed.

- The Charm EZ monitors the assay progress during the incubation.
- Damaged strips are detected by Charm EZ and will result in an error message.
- Remove the strip and restart the assay.

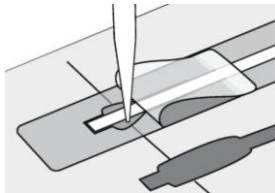
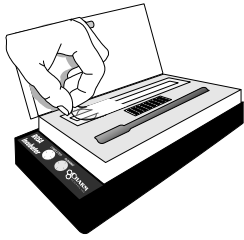
Sample and Test Information

- Raw, commingled cow milk must be refrigerated or cool (0 to 15°C) for testing.
- Test refrigerated samples within 5 days of milking.
- To preserve samples or Controls after testing, freeze samples at -15°C or below. See **Long-Term Sample and Control Storage**.
- Test may be performed at ambient temperatures of 10 to 30°C in naturally circulating air.

SL-KIWI Beta-lactam Test for Milk Procedure with ROSA Incubator

Check that ROSA Incubator temperature is $56 \pm 1^\circ\text{C}$.

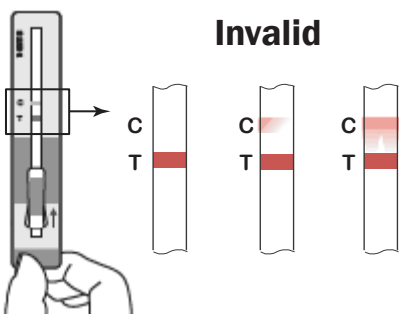
Use SL-KIWIP test strips. Re-shape dented sample compartments to fit into ROSA Incubator.



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|---------------|--|
| Step 1 | <ul style="list-style-type: none"> • Mix all samples well before testing. |
| Step 2 | <ul style="list-style-type: none"> • Label test strip(s) with sample identification. Avoid crushing sample compartment. |
| Step 3 | <ul style="list-style-type: none"> • Place test strip in ROSA Incubator. • Holding test strip flat in ROSA Incubator, use TAB to expose sample compartment by peeling tape back to "Peel to Here" line. Avoid lifting the test strip and sponge under tape. |
| Step 4 | <ul style="list-style-type: none"> • Using 300 μl pipet, draw up sample. Avoid foam and bubbles. • Holding pipet vertically, slowly pipet 300 μl ($\pm 15 \mu\text{l}$) sample or Control into sample compartment at ROSA Incubator indicator line (as shown). |
| Step 5 | <ul style="list-style-type: none"> • Reseal tape over sample compartment. • When performing multiple tests in a ROSA Incubator: <ul style="list-style-type: none"> ○ Peel, pipet and reseal before starting next test strip. ○ Complete all test strips within 1 minute. |
| Step 6 | <ul style="list-style-type: none"> • Close lid on ROSA Incubator and latch. Timer starts and red light illuminates. |
| Step 7 | <ul style="list-style-type: none"> • Incubate for 8 minutes, but not more than 10 minutes. • At 8 minutes, a beeper and alternating yellow and red blinking lights start. |
| Step 8 | <ul style="list-style-type: none"> • Remove test strip(s) from ROSA Incubator. Do not squeeze sample compartment. Hold test strip with sample compartment in the down position until interpreted. • Read within 5 minutes of incubation completion. • Lower ROSA Incubator lid. Do not re-latch. |

Visual Inspection

Hold test strip vertically with sample compartment in down position. Do not squeeze the sample compartment. Wipe foreign matter (dust, etc.) off test strip.



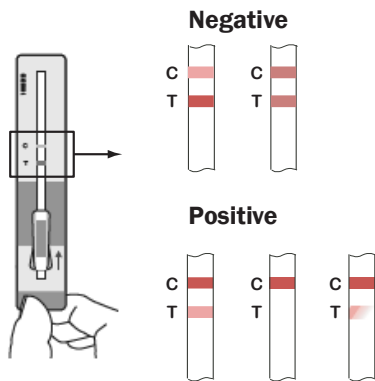
The test is **INVALID** if any of the following are observed:

- **C** (Control) line is missing.
- **C** line is smeared or uneven.
- **C** or **T** (Test) line is obscured by sample or Control.
- Beads do not flow past **T** or **C** lines.

Re-test INVALID samples. DO NOT INTERPRET OR PUT INVALID TEST STRIPS IN ROSA READER.

Interpretation

Visual Interpretation



Negative - T (Test) line is **same as or darker** than C (Control) line.

Positive - T line is clearly **lighter** than C line, or T line is absent, or partially or unevenly colored. Sample should be retested. See **Retest of Initial Positive**.

ROSA Reader Interpretation - The ROSA Reader is an optional feature.



Within 5 minutes of completing incubation, insert clean and visually valid test strip into ROSA Reader as shown. Slide test strip completely into slot until it stops.

Read results on **SLBL** channel. If desired, enter **SAMPLE** and/or **OPERATOR**. Press **ENTER** to read. A numerical value (**READING**) and an interpretation (**RESULT**) are displayed on screen.

Results are stored in memory and can be recalled to the display and downloaded to printer or computer.



Negative - If **READING** is a negative number or zero, the ROSA Reader will display **RESULT NEGATIVE**. Report as "Not Found."

Positive - If **READING** is a positive number, the ROSA Reader will display **RESULT POSITIVE**. Sample should be retested. See **Retest of Initial Positive**.

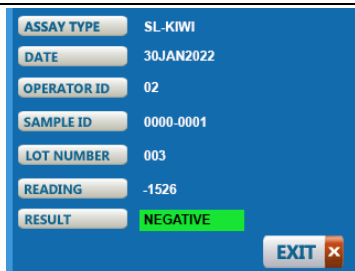
Charm EZ Interpretation (Read only mode) - The Charm EZ is an optional feature.



Within 5 minutes of completing incubation, insert clean and visually valid test strip into Charm EZ as shown. Slide test strip completely into slot until it stops.

If desired, enter **SAMPLE ID**, **LOT NUMBER**, and/or **OPERATOR ID**. Close door to read. A numerical value (**READING**) and an interpretation (**RESULT**) are displayed on screen.

Results are stored in memory and can be recalled to the display and downloaded to printer or computer.



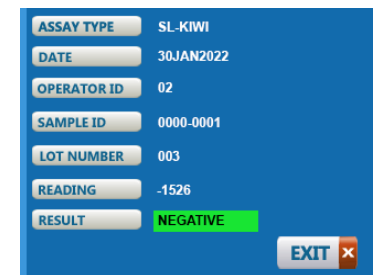
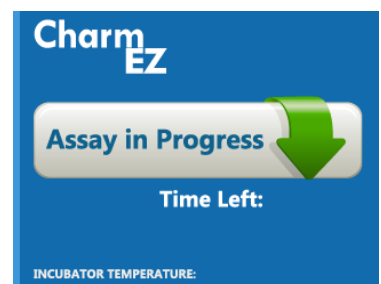
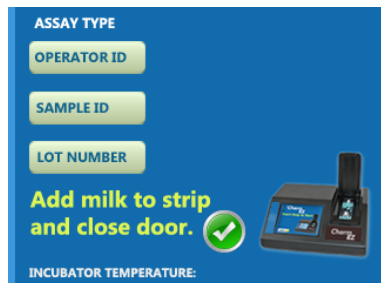
Negative - If **READING** is a negative number or zero, the Charm EZ will display **RESULT NEGATIVE**. Report as "Not Found."

Positive - If **READING** is a positive number, the Charm EZ will display **RESULT POSITIVE**. Sample should be retested. See **Retest of Initial Positive**.

SL-KIWI Beta-lactam Test for Milk Procedure with Charm EZ Incubation or EZProtect System

Use SL-KIWI test strips. Re-shape dented sample compartments to fit into Charm EZ.

*For use with Charm EZProtect System – please contact Charm Sciences for further instructions.



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| Step 1 | <ul style="list-style-type: none"> Power up Charm EZ and wait for Insert Strip to Start screen. |
| Step 2 | <ul style="list-style-type: none"> Mix all samples well before testing. |
| Step 3 | <ul style="list-style-type: none"> Label test strip(s) with sample identification. Avoid crushing sample compartment. |
| Step 4 | <ul style="list-style-type: none"> Place test strip in Charm EZ. The Charm EZ will automatically read the test strip and adjust the Assay Type and incubator to the required temperature. Wait for incubator temperature check to turn green. |
| Step 5 | <ul style="list-style-type: none"> Tap the touch screen to enter the Operator ID, Sample ID or Lot Number information. |
| Step 6 | <ul style="list-style-type: none"> Holding test strip flat in Charm EZ, use TAB to expose sample compartment by peeling tape back to “Peel to Here” line. Avoid lifting the test strip and sponge under tape. |
| Step 7 | <ul style="list-style-type: none"> Using 300 µl pipet, draw up sample. Avoid foam and bubbles. Holding pipet vertically, slowly pipet 300 µl (± 15 µl) sample or Control into sample compartment at indicator line (as shown). |
| Step 8 | <ul style="list-style-type: none"> Reseal tape over sample compartment. |
| Step 9 | <ul style="list-style-type: none"> Close lid on Charm EZ. This will start the 8 minute timer. Do not open the door while the assay is in progress. |
| Step 10 | <ul style="list-style-type: none"> Assay Results will appear on the screen automatically at the end of the incubation period. Remove test strip from Charm EZ to return to the Insert Strip to Start Screen. |

Retest of Initial Positive

1. Test "initial positive" samples in duplicate along with one Negative Control and one Positive Control.
2. Negative Control must be **RESULT NEGATIVE** with **READING** less (more negative) than -600 on ROSA Reader or Charm EZ.
3. Positive Control must be **RESULT POSITIVE** with **READING** greater than +400 on ROSA Reader or Charm EZ.
4. If either Control is not within range, repeat testing of "initial positive" with Controls. If either Control is still not within range after retest, discontinue testing and contact Charm Sciences.
5. If both Controls are within range and either or both of the retested samples are **RESULT POSITIVE**, the sample is a "SL-KIWI Beta-lactam Positive Test."

Order Codes and Kit Contents

KITS	NOT SUPPLIED WITH KIT
<p>LF-SL-KIWIP-40NSK:</p> <ul style="list-style-type: none"> • (1) container of 40 SL-KIWIP test strips • (1) Operator's Manual <p>LF-SL-KIWIP-100K:</p> <ul style="list-style-type: none"> • (1) container of 100 SL-KIWIP test strips • (5) Penicillin G Positive Tablets • (1) Operator's Manual <p>LF-PG-1-TBL:</p> <ul style="list-style-type: none"> • (1) Penicillin G Positive Tablet 	<p>1-MLT-96 (box of 96 disposable pipet tips, 200-1000 µl)</p> <p>1-MLT-960 (10 boxes of 96 disposable pipet tips, 200-1000 µl)</p> <p>1-MLT-BG (bag of 1,000 disposable pipet tips, 200-1000 µl)</p> <p>LF-ROSA-PIPET-500 (500 disposable ROSA-pipets, 300 µl)</p> <p>LF-ROSA-PIPET-5000 (5000 disposable ROSA-pipets, 300 µl)</p> <p>PIP-300UL-1STOP-M (300 µl fixed volume pipet)</p> <p>PIP-100-1000UL-1STOP (100 to 1000 µl pipet)</p> <p>LF-INC4-ADJUSTABLE (4 place ROSA incubator with adjustable time and temperature)</p> <p>LF-ROSA-EZ (Charm EZ System)</p> <p>LF-ROSA-EZPROTECT (Charm EZProtect System)</p>

Warranty Information

Charm Sciences, Inc. ("Charm") warrants each reagent product, including but not limited to test kits, to be free from defects in materials and workmanship and to be free from deviations from the specifications and descriptions of Charm's reagent products appearing in Charm's product literature, when stored under appropriate conditions and given normal, proper and intended usage, until the expiration of such reagent product's stated shelf life, or, if none is stated, for one year from the date of delivery of such reagent product to the end-user purchaser. **THIS WARRANTY IS IN LIEU OF ALL OTHER WARRANTIES, WHETHER STATUTORY, EXPRESS, IMPLIED (INCLUDING WARRANTIES OF TITLE, NON-INFRINGEMENT, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE AND ALL WARRANTIES ARISING FROM COURSE OF DEALING OR USAGE OF TRADE).** The warranty provided herein may not be altered except by express written agreement signed by an officer of Charm. Representations, oral or written, which are inconsistent with this warranty are not authorized and if given, should not be relied upon. In the event of a breach of the foregoing warranty, Charm's sole obligation shall be to replace any reagent product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Charm promptly of any such defect prior to the expiration of said warranty period. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Charm is willing to replace any nonconforming reagent product or part. **Charm shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damages sustained by any customer from the use of its reagent products.** Except for Charm's obligation set forth above to replace any reagent product that proves defective within the warranty period, Charm shall not be liable for any damages of any kind arising out of or caused by any incorrect or erroneous test results obtained while using any such reagent product, whether or not caused by a defect in such reagent product.