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QUIDEL

R-Mix Too™ FreshFrozenCells

Ampoules of frozen cells for use in the preparation of Cultured Cell Shell- Vials and Multi-well Plates

For *in vitro* diagnostic use.

A symbols glossary can be found at quidel.com/glossary.

INTENDED USE

R-Mix Too FreshFrozenCells [Madin-Darby canine kidney (MDCK) and human lung carcinoma (A549)] ampoules are intended to be used in the production of cultured cells; monolayers in shell-vials or multi-well plates.[1,2] Although cultured cells may be purported to be useful for virus isolation, frozen cells must first be propagated (cultured) prior to this use. The laboratory must determine the cell type to be used as host for isolation of a particular virus.[3] Use for diagnostic procedures without prior culture has not been established.

FreshFrozenCells products are provided as ampoules of cells frozen in a cryoprotective solution [DMSO in a base of Minimum Essential Medium (MEM) and fetal bovine serum (FBS)].

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Thawed FreshFrozenCells product cannot be re-frozen.
- As with all methods for virus identification using cultured cells, personnel must be properly trained in virus culture and safe handling techniques,[4,5] i.e., manipulations which present potential personnel hazards should be conducted in a Class II biosafety cabinet; and gloves should be worn at all times.
- Although cultured cells may be purported to be useful for virus isolation, frozen cells should first be propagated (cultured) prior to their use. R-Mix Too cells should be cultured in their final container (i.e., shell-vials, multi-well plates, tubes or flasks).
- Cultured cells used for virus identification may also support the replication of infectious agents which are classified by the CDC as agents requiring cultivation under BSL-3 conditions.[6] Consult CDC for listing of the BSL-3 infectious agents and recommendations.
- The expiration date stated on the package is correct if product is maintained constantly at −70°C or colder. Any deviations in conditions of storage or thawing can cause diminished product quality prior to the expiration date.
- Cultures and specimens should be autoclaved or disinfected with a solution of sodium hypochlorite (1:10 final dilution of household bleach) prior to disposal.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.

- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STABILITY AND STORAGE INSTRUCTIONS

Indications of instability or deterioration

- Due to variations in end-user mechanical freezer storage temperatures, cells may potentially lose some viability during storage. This loss may lengthen the time required for the planted cells to reach confluence.
- To insure viability of the cells, it is imperative that they remain frozen until immediately prior to use.
- Should the following characteristics or indicators of deterioration be observed during the culturing of the cells, the cell cultures should be discarded and the distributor contacted:
  - Failure of the planted cells to reach confluence
  - Changes in characteristic cell morphology, e.g., rounding, sloughing, retraction, or vacuolization
  - Turbid or yellow (indicating an acidic pH change) culture medium (indicative of bacterial or fungal contamination).

Storage Instructions

- FreshFrozenCells maintained at −70°C or lower have a shelf-life of 6 months from date of shipment from Quidel facilities.
- R-Mix Too FreshFrozenCells are shipped on dry ice. Upon receipt, some dry ice **must** cover the box containing the ampoule(s). It is imperative that product does not warm above −70°C. Little or no dry ice present in the package upon receipt is indicative that the cells may have warmed or thawed and lost their viability. Please contact the distributor for further instructions.
- Upon receipt, rapidly transfer the ampoules to a mechanical freezer (preferably a chest-type model) that is maintained at −70°C or lower, or to the vapor phase of a Dewar-type liquid nitrogen storage container without allowing them to warm or thaw (for storage up to the expiration date of the product at −70°C or lower).

QUALITY ASSURANCE

- R-Mix Too cells are from reliable, reputable, and trackable sources. Prior to acceptance into Quidel's production facility, the cell type is reviewed through documentation history and laboratory analysis to verify that no microorganisms (by sterility testing) and no viruses [as evidenced by the absence of cytopathic effect (CPE)] are known to be present.
- **Note concerning cell lines of human origin:** *Quidel's stock inventories of this cell line of human origin have been tested to verify the absence of HIV and HBV viral DNA using PCR techniques.*

Lot Specifications

- Prior to shipment of each R-Mix Too FreshFrozenCells lot, representatives of the lot are:
  - Screened for the absence of *Mycoplasma spp*. and other adventitious microorganisms.
  - Planted and the resulting monolayers examined microscopically for morphology, confluence, and uniformity.
  - Characterized as to species identity by isoenzyme analysis.
- Information beyond that provided by the Product Insert, Lot Specification Sheet, or Material Safety Data Sheet is available upon request.

LIMITATIONS

Conditions encountered during shipment may affect the shelf life of frozen cell culture ampoule product. To minimize this effect, do not allow stored ampoules to warm or thaw (store at –70°C or lower) prior to preparation of shell-vials or multi-well plates. Once propagated, all cultures should be examined for

appearance and morphology prior to inoculation. Aging of cell cultures due to passage can result in the loss of sensitivity to virus isolation and replication.

PRELIMINARY COMMENTS

Cultured cells provide the necessary living host systems for the isolation of viruses. The viral isolation procedure typically involves incubating a prepared clinical specimen with an appropriately permissive cell line (Table 1).[7,8] This incubation period is variable and dependent upon the virus. The classic detection method for viral infection in cell culture is the observation of cellular changes due to the infection and replication of the virus, termed cytopathic effect (CPE). In recent years, the use of monoclonal antibodies against antigens specific to an infectious agent to determine the agent's presence and identity has become widely accepted. This methodology has increased the sensitivity of the cell culture system and decreased the time to agent detection.

Table 1. R-Mix Too FreshFrozenCells and their virus susceptibility profiles

REF	Cell Line/Origin	Infectious Agents
97-	R-Mix Too MDCK and A549* (canine kidney and human lung carcinoma*)	influenza A and influenza B and adenovirus, HSV, influenza, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.
Package Format Suffix		
-00050C	"50-mer" ampoule [Sufficient for approximately 50 wells of a 24 size multi-well plate or 50 shell-vials/13 mm tubes.]	
Additional cell types or formats may be available on request.		

SUGGESTED PROCEDURE FOR USE

Thawing of Cells

1. All cell culturing activities should be conducted using aseptic techniques.
2. Pre-warm *Cell Planting Medium* (10-200100) in a 35˚C to 37˚C water bath.
3. Thaw R-Mix Too FreshFrozenCells ampoule rapidly with a gentle swirling motion in a 35˚C to 37˚C water bath.
   - Do not allow the ampoule to thaw longer than 4-minutes.
   - Do not submerge ampoule below the cap juncture since water bath water can be drawn into the ampoule and contaminate the cells.
4. Decontaminate the submerged portion of the ampoule with alcohol wipes (or similar product). Only the portion of the ampoule submerged must be decontaminated. Do not allow the alcohol to seep under the cap of the ampoule. Introduction of alcohol into the ampoule will have detrimental effects on the cells.
5. Mix the thawed cells by pipetting up and down 2 to 3 times using a sterile transfer pipette. DO NOT VORTEX.

Preparation of Shell-vials or 24-well Multi-well Plates

1. Transfer 49.5 mL of the warmed *Cell Planting Medium* (10-200100) to a sterile container.
2. Transfer the entire contents of the ampoule using a sterile transfer pipette to the pre-warmed *Cell Planting Medium*.
3. Mix the cells and medium by inversion or gentle swirling for approximately 1 minute. DO NOT VORTEX.
4. Transfer 1 mL of cell mixture into each shell-vial or well of a sterile 24-well multi-well plate. Prepare 50 wells in this manner.
5. Re-cap each shell-vial tightly and place into the stationary rack. The 24-well plates should be stored in a 35˚C to 37˚C , 5% CO_2, humidified incubator.

6. Incubate at 35°C to 37°C, 5% CO_2, humidified incubator for a minimum of 3 days. Care should be taken not to disturb the cells for the first 48 hours.
7. Examine the monolayers for confluency. A confluency of 90% to 100% is acceptable for use in most cell cultures.

Specimen Inoculation

1. Remove *Cell Planting Medium* by aspiration. If planting medium is removed by decanting, the R-Mix Too cells should be rinsed once with 1 mL of RM-03T R-Mix Refeed Medium (10-330100).
2. Add 1 mL of RM-03T R-Mix Refeed Medium to shell-vial or well respectively.
3. Add prepared sample according to established SOP (standard operating procedure).
4. Process shell-vials or multi-well plates according to established SOP.
5. Monolayers should be examined 24 hours post-inoculation or at recommended intervals for the presence of cytopathic effect.

RESULTS

Refer to appropriate reference material for expected results and reporting suggestions.

QUALITY CONTROL

Non-inoculated cell controls should be run with each batch of specimens tested for virus to serve as negative controls. Negative controls are handled the same as inoculated monolayers.

Positive virus controls may be run using previously identified viral agents that will produce the desired result from a positive patient sample (i.e., CPE).

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	 Biological risks
IVD For *In Vitro* diagnostic use	CONT Contents/Contains