DIAGNOSTIC HYBRIDS
A Quidel Company

2005 East State Street, Suite 100
Athens, OHIO 45701 USA
Phone: +1(800) 874-1517
Fax: +1(740) 592-9820
www.dhiusa.com

Fresh*Frozen*Cells®

Ampoules of frozen cells for use in the preparation of Cultured Cell Flasks, Shell-vials, and Multi-well Plates.

For *in vitro* Diagnostic Use

Please contact Diagnostic Hybrids Technical Support for technical assistance regarding this procedure.

USA Toll-free: (800) 874-1517
Outside the US: +1(858) 552-1100
Fax: +1(740) 592-9820
E-mail: technicalsupport@quidel.com

Symbols Lexicon/Glossary

IVD	In Vitro Diagnostic Medical Device	LOT	Batch code/lot number
	Temperature limit	REF	Catalog number
	Consult e-labeling instructions for use		Use by YYYYMONDD
	Patent Numbers		Manufacturer
CE	CE mark of conformity (Conformité Européen)	EC REP	Authorized representative in the European Community

I. Intended Use

Diagnostic Hybrids **Fresh*Frozen*Cells**® ampoules are intended to be used in the production of cultured cells; monolayers in flasks, shell-vials, multi-well plates, or tubes. Although cultured cells may be purported to be useful for virus or *Chlamydia* 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 and/or *Chlamydia*. Use for diagnostic procedures without prior culture has not been established. **Fresh*Frozen*Cells** ampoules are provided in the following product formats (Table 1):

- *Singles* – a single cell line
- *MixedCells™ (Patented)* – two cell lines mixed at approximately equal cell density

Fresh*Frozen*Cells 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)].

II. Warnings and Precautions

A. For *in vitro* diagnostic use.
B. Thawed **Fresh*Frozen*Cells**® product cannot be re-frozen.
C. As with all methods for virus identification using cultured cells, personnel must be properly trained in virus culture and safe handling techniques as described in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, 2007, 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.
D. Although cultured cells may be purported to be useful for virus or Chlamydia isolation, frozen cells should first be propagated (cultured) prior to their use. *MixedCells*™ (R-Mix™, R-Mix Too™, Super E-Mix™) cells should be cultured in their final container (i.e., shell-vials, multi-well plates, tubes or flasks).
E. Cultured cells used for virus or *Chlamydia spp*. identification may also support the replication of infectious agents which are classified by the CDC as agents requiring cultivation under BSL-3 conditions. Consult CDC for listing of the BSL-3 infectious agents and the CDC recommendations.
F. The expiration date stated on the package is correct if product is maintained constantly at -70ºC or colder. Any deviations in conditions of storage or thawing can cause diminished product quality prior to the expiration date.
G. Cultures and specimens should be autoclaved or disinfected with a solution of sodium hypochlorite (1:10 final dilution of household bleach) prior to disposal.

III. Stability and Storage Instructions

Indications of instability or of deterioration

A. 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.
B. To insure viability of the cells, it is imperative that they remain frozen until immediately prior to use. **NOTE**: See section **VII.B, Thawing of Cells** for process and precautions.
C. 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

A. **Fresh*Frozen*Cells** maintained at -70°C or lower have a shelf-life of 6-months from date of shipment from Diagnostic Hybrids (DHI) facilities. **NOTE**: Refer to original ampoule container (box) for expiry date; due to the design and inventory control of this frozen product, the same lot can have different expiry dates because shelf-life is determined from the date of shipment from the DHI facility.
B. **Fresh*Frozen*Cells** are shipped on dry ice. Upon receipt, some dry ice **must** still 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.
C. Upon receipt, rapidly transfer the vials 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).

IV. Quality Assurance

A. Cells are from reliable, reputable, and traceable sources. Prior to acceptance into DHI'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.
B. *Note concerning cell lines of human origin: DHI's stock inventories of the cell lines of human origin have been tested to verify the absence of HIV and HBV viral DNA using PCR techniques.*

Lot Specifications

A. Prior to shipment of each **Fresh*Frozen*Cells** 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
B. Product Insert, Lot Specification Sheet, or Material Safety Data Sheet is available upon request.

V. 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 flasks 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.

VI. 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). 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). The use of monoclonal antibodies against antigens specific to an infectious agent to determine the agent's presence and identity prior to the appearance of CPE has become widely accepted. This methodology has increased the sensitivity of the cell culture system and decreased the time to agent detection. Investigators have discovered the benefits derived from mixing two different cell lines to form a single monolayer. Combining two cell lines allows for detection of agents cultivated in each of the individual cell lines to be detectable in a single container. When combined with centrifugation-enhancement methods and detection of early viral antigens, mixed monolayers can reduce the number of individual cell culture units used to detect a broader range of viruses in a significantly shorter length of time.

VII. Suggested Procedure for Use

A. Cell Planting Medium: Preparation of the planting medium should be conducted using aseptic techniques.

1. Thaw the frozen *Supplement for Cell Planting Medium* (10-200100 EU1), with a gentle swirling motion in a pre-warmed 35° to 37°C water bath. Do not submerge the vial below the juncture of the cap since water bath water can be drawn into the vial and contaminate the cells. Do not allow the concentrate to remain in the water bath for longer than 10-minutes.
2. Aseptically transfer the entire volume of the concentrate (10-200100 EU1) to the bottle of *Cell Planting Medium Base* (10-200100 EU2) with a 10-mL pipette.
3. Mix well by gentle swirling.
4. Label as *Cell Planting Medium* with the date prepared, preparer's initials, and a 4-month expiration date. Store at 2° to 8°C.

B. Thawing of Cells

1. All cell culturing activities should be conducted using aseptic techniques.
2. Pre-warm *Cell Planting Medium* in a 35° to 37°C water bath.
3. Thaw **Fresh*Frozen*Cells** ampoule rapidly with a gentle swirling motion in a 35° to 37°C water bath.
   - Do not allow the ampoules 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.

C. Preparation of Shell-vials, Tubes, or 24-well Multi-well Plates

1. Transfer the entire contents of the ampoule using a sterile transfer pipette to a sterile container containing 49.5-mL of pre-warmed *Cell Planting Medium*.
2. Mix the cells and medium by inversion or gentle swirling for approximately 1-minute. DO NOT VORTEX.
3. Add 1-mL of diluted cells to each shell-vial, 13-mm tube or well of the 24-well plate with a sterile pipette; use 2-mL for each 16-mm tube. Make certain that the cell suspension remains mixed during planting to ensure an even and adequate cell distribution to each vessel.
4. Re-cap each shell-vial tightly and place into the stationary rack. The 24-well plates should be stored in a 35° to 37°C, 5% CO_2, humidified incubator.
5. Incubate at 35° to 37°C for a minimum of 3-days. Care should be taken not to disturb the cells for the first 48-hours.
6. Examine the monolayers for confluency. A confluency of 90% to 100% is acceptable for use in most cell culture.

D. Preparation of T-75 cm^2 flasks

1. Transfer 70-mL of the warmed (35° to 37°C) *Cell Planting Medium* to a sterile T-75 cm^2 flask.
2. Transfer the entire contents of the ampoule using a sterile transfer pipette to the T-75 cm^2 flask of pre-warmed *Cell Planting Medium* and gentle swirling. DO NOT VORTEX.
3. Incubate at 35° 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.
4. Examine the monolayers for confluency. A confluency of 90% to 100% is acceptable for use in most cell culture.

E. 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 appropriate refeed medium to flask, shell-vial, or well respectively.
3. Add prepared sample according to established SOP (standard operating procedure).
4. Process shell-vials or plates according to established SOP.
5. Monolayers should be examined 24-hours post-inoculation or at recommended intervals for the presence of cytopathic effect (CPE).

VIII. Results

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

IX. 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).

X. References

1. Viral Culture; Approved Guideline M41-A. Vol. 26, No. 7. Clinical and Laboratory Standards Institute, Wayne, PA. 2006.
2. Jacob J.P., C.M. Jones, J.P. Baille. Characteristics of a human diploid cell designated MRC5. Nature, 1970, 227: p 168 -170.
3. Holper, J.C. Characteristics of primary cultures and diploid cells - Technology of production. National Cancer Institute Monograph. 1968 No. 29 p 21-31.
4. McAteer J.A., W.H.J. Douglas. Monolayer culture techniques in: W.B. Jakoby (ed): Cell culture Methods in Enzymology, 1979, Vol. 58, p 132-140, Academic Press.
5. Razonable, R.R., Paya, C.V., Smith, T.C. Role of the Laboratory in Diagnosis and Management of Cytomegalovirus Infection in Hematopoietic Stem Cell and Solid-Organ Transplant Recipients. J. Clin. Microbiol. 2002 vol. 40: 746-752.
6. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, 2007, CDC-NIH manual. [http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm]
7. Biosafety Manual, 3rd edition, 2004. World Health Organization [Manual is available in additional languages; refer to WHO web page [http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/]
8. Laboratory Biosafety Guidelines, 3rd edition, 2004. Published by authority of the Minister of Health, Population and Public Health Branch, Centre for Emergency Preparedness and Response [Guideline is available in French or English; refer to web page [http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/index.html]

Warranty Statement

These products are warranted to perform as described in their labeling and the Diagnostic Hybrids literature when used in accordance with their instructions. THERE ARE NO WARRANTIES WHICH EXTEND BEYOND THIS EXPRESS WARRANTY AND DIAGNOSTIC HYBRIDS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. Diagnostic Hybrids sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Diagnostic Hybrids to repair or replace the products.

TABLE 1: Cell Culture Types

Catalog No.	Cell Types	Tissue Source	Infectious Agents
Singles Cell Line Prefixes *[Cell types of human origin are indicated with asterisks (*) in table below.]*			
51-	MRC-5*†	human embryonic lung	CMV, HSV, adenovirus, influenza, mumps, echovirus, poliovirus, rhinovirus, RSV, VZV
53-	BGMK	Buffalo green monkey kidney	*Chlamydia*, HSV, coxsackie B, poliovirus.
56-	A549*†	human lung carcinoma	adenovirus, HSV, influenza, MPV, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV
62-	MNA	mouse neuroblastoma	rabies
76-	RD*	human rhabdomyosarcoma	adenovirus, echovirus, hsv, poliovirus
83-	MDCK	canine kidney	influenza A, influenza B, some types of adenovirus, reovirus, coxsackie virus
84-	Vero	African green monkey kidney	adenovirus, coxsackie B, HSV, measles, mumps, poliovirus type 3, rotavirus, rubella
44-	MRHF*†	human foreskin fibroblast	adenovirus, CMV, echovirus, HSV, mumps, poliovirus, rhinovirus, VZV
MixedCells™ Prefixes			
92- Super E-Mix™	BGMK-hDAF and A549*	genetically modified Buffalo green monkey kidney and Human lung carcinoma	HSV, coxsackie B, coxsackie a, echovirus, and poliovirus. and adenovirus, HSV, influenza, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.
96- R-Mix™†	Mv1Lu and A549*	mink lung and human lung carcinoma	HSV, CMV, influenza A, influenza B and adenovirus, HSV, influenza, MPV, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.
97- R-Mix Too™	MDCK and A549*	canine kidney and human lung carcinoma	influenza A, influenza B and adenovirus, HSV, influenza, MPV, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.
Package Format Suffix			
-00050	"50-mer" vial *[Sufficient for approximately* 50*-wells of a 24 size multi-well plate or* 50 *shell-vials/13*-mm *tubes or* 25 *16-mm tubes or* one *T-75 cm² flask.]*		
Additional cell types or formats may be available on request.			

† Annex II, List B associate products; McCoy (Chlamydia) and MRC-5 (CMV)

TABLE 2: Example of *MixedCells*™ Appearance Note: Mixed cell preparations should have a dual cell appearance.

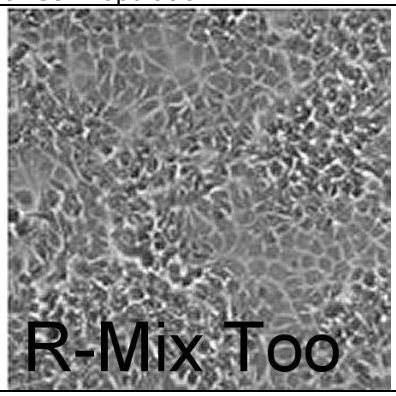
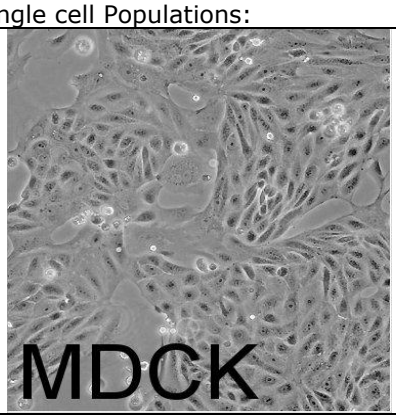
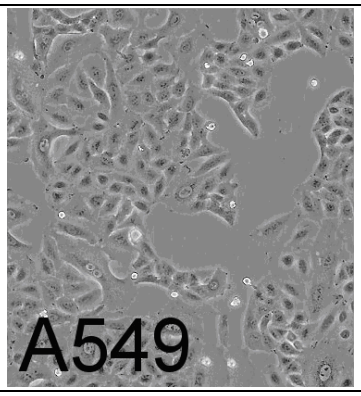
Dual Cell Population:	Single cell Populations:	
		

TABLE 3: Cell Culture Media Products

Catalog No. (100 and 500-mL)		Description
10-200100		Cell Planting Medium
10-200100 EU1		Supplement for Cell Planting Medium Base (10-200100 EU2)
10-200100 EU2		Cell Planting Medium Base
10-210125		MRC-5 Cell Growth Medium
10-290030		TurboTreat® (A Mink Lung Cell Pretreatment Medium)
10-320100	10-320500	RM-02 Refeed Medium (2% FBS)
10-330100	10-330500	RM-03T R-Mix™ Refeed Medium
10-350100	10-350500	RM-05 Refeed Medium (10% FBS)
10-360100		Zero-Serum Refeed Medium – PS
10-380100	10-380500	Super E-Mix™ Refeed Medium
10-390100	10-390500	Zero-Serum Refeed Medium – PSGA