

ReadyCells®

Singles and Mixed

Frozen Cell Monolayers in Shell-vials
Cultured cells for use in virus and/or *Chlamydia*
isolation.

For *in vitro* Diagnostic Use

Please contact Quidel Corporation 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

ReadyCells® Singles and Mixed are covered by U.S. Patent Nos. 6,376,172; 6,406,842; 6,573,080; 6,946,291; 6,472,206; 7,341,829; 6,821,741; 7,186,504; others pending

R-Mix™ is covered by U.S. Patent Nos. 6,168,915; 6,376,172; 6,406,842; other patents.

R-Mix Too™ is covered by U.S. Patent No. 6,946,291.

Super E-Mix™ is covered by U.S. Patent No. 6,168,915; 6,573,080; 6,821,741; 7,186,504; others pending

Symbols Lexicon/Glossary

	Temperature limit		Batch code/lot number
	Use by YYYY-MON-DD		Catalog number
	Consult e-labeling instructions for use		
	Patent Numbers		Manufacturer
	Authorized representative in the European Community		CE mark of conformity (Conformité Européen)

I. Intended Use

Diagnostic Hybrids **ReadyCells**® are frozen cultured cell monolayers intended for virus and/or *Chlamydia* isolation and to aid in the diagnosis of diseases associated with infectious agents. These cultured cells should not be used for serial propagation. The laboratory must determine the cell type to be used as host for isolation of a particular virus and/or *Chlamydia*. **ReadyCells** are provided in the following product formats (see Table 1):

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

II. Summary and Explanation

Specific cultured cell types provide the necessary living host systems for the identification of viruses and *Chlamydia spp.* Such cultured cells are used in the isolation, detection and identification of these infectious agents.^{1,2} The procedure typically consists of incubating a specimen with an appropriately sensitive cultured cell type. This incubation period is variable and is dependent on the detection system used. The classic detection method is the observation of cellular changes due to infection of the cultured cells, termed cytopathic effect (CPE). The use of monoclonal antibodies against specific infectious agents to confirm an agent's identity has become widely accepted; this method has increased the sensitivity of the cultured cell system and substantially decreased the time to infectious agent detection.

Diagnostic Hybrids **ReadyCells** frozen cell monolayers expand the utility of cultured cells by providing laboratories greater flexibility. Cultured cell monolayers are cryopreserved at optimum confluency and sensitivity. They are supplied to the laboratory ready to thaw, reseed, and use.

¹ Viral Culture; Approved Guideline M41-A. Vol. 26, No. 7. Clinical and Laboratory Standards Institute, Wayne, PA, 2006.

² 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.

III. Warnings and Precautions

1. For *in vitro* diagnostic use.
2. **ReadyCells** must be received frozen and completely covered on all sides with dry ice. Dispose of dry ice in a safe manner according to your facility's policies.
3. Thawed **ReadyCells** product cannot be re-frozen.
4. Cultured cells should be used (inoculated) on or prior to their labeled expiration date.
5. 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,^{3,4} *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.
6. Cultured cells used for virus or *Chlamydia spp.* detection may also support the replication of infectious agents which are classified by the CDC as agents requiring cultivation under BSL-3 conditions. Please consult <http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm> for a listing of the BSL-3 infectious agents and the CDC recommendations.
7. Cultures and specimens should be autoclaved or disinfected with a solution of sodium hypochlorite (1:10 final dilution of household bleach) prior to disposal.

IV. Materials Provided

1. **ReadyCells** are provided as single or mixed cultured cell monolayers adhered to glass coverslips contained in shell-vials. They are available in a configuration of 24 shell-vials per box.
2. **ReadyCells** products and their susceptibility to specific infectious agents are listed in Table 1.
3. The cultured cells used in **ReadyCells** are characterized by isoenzyme analysis and have been tested and found free of *Mycoplasma spp.* and other adventitious organisms. The current passage number of each cell type in the product is noted on the Lot Specification Report, which is supplied with each shipment of **ReadyCells** and which is also available upon request.

V. Materials Required but Not Provided

1. It is strongly recommended that the **ReadyCells** dry heat block be used to thaw the frozen monolayer cultures.
2. It is strongly recommended that the appropriate culture medium ("Refeed Medium", available from Diagnostic Hybrids) is used.
3. For the products, R-Mix and R-Mix Too, a wash solution ("Rinse Buffer", available from Diagnostic Hybrids) is recommended to be used immediately after thawing the product.

VI. Storage Instructions

1. Upon receipt, rapidly transfer the **ReadyCells** monolayers from the dry ice shipping container directly to the final storage freezer without delay.
2. **ReadyCells** must be stored at -70° C or colder upon receipt.
3. Do not store the **ReadyCells** monolayers in liquid nitrogen.

VII. Quality Control

1. The **ReadyCells** monolayers should be examined, upon thawing and refeeding, for integrity of the culture cell monolayer. The monolayer should be intact and cover the surface of the coverslip; it may not appear as a solid sheet.
2. Negative controls should be run with each batch of specimens tested for virus. Negative controls consist of non-inoculated monolayers and are handled the same as the inoculated monolayers.

VIII. Limitations

1. **ReadyCells** should be thawed for use on or before their labeled expiration date.
2. **ReadyCells**, once thawed, should be reseed according to these instructions, and maintained at 35° to 37°C until used, but not longer than 8-hours.

³ Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, 2007, CDC-NIH manual.

[<http://www.cdc.gov/od/ohs/biosfty/bmb15/bmb15toc.htm>]

⁴ 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_CS_R_LYO_2004_11/en/]]

IX. Procedure

A. Preliminary Comments and Precautions

1. Warm refeed medium to 25° to 37°C before adding to cultures.
2. Do not disturb the cell monolayers during the thawing process.
3. Cells must not be allowed to dry at any stage during the cultivation process.
4. Use a fresh, sterile pipette for each specimen to avoid cross contamination.
5. When inoculating a specimen into a culture, be careful to not splash the residual liquid from the pipette, since it could contaminate adjacent cultures.

B. Thawing and Refeeding Cell Cultures

1. Verify that the **ReadyCells** heat block has reached a steady temperature of 37°C.
2. Remove appropriate number of **ReadyCells** shell-vials from the freezer and **immediately** transfer to the heat block. (**Note:** The heat block should be in close proximity to the freezer. If the distance is excessive [greater than 45-seconds transit time] the **ReadyCells** should be transported to the heat block on dry ice.)
3. Incubate shell-vials for 4-minutes. (**Note:** Incubation greater than 4-minutes may cause monolayer deterioration.)
4. Aspirate the freeze medium, taking care not to damage the monolayers, and collect the medium in a disinfectant-containing trap. **Do not let cells dry.**

Note 1: For the products R-Mix/R-Mix Too ReadyCells, perform steps A), B), C), and D), below.

Note 2: For all others, go directly to step Z).

- A) Add 0.5-mL of R-Mix™ **ReadyCells**® Rinse Buffer to each shell-vial.
- B) Incubate at room temperature for 4-minutes.
- C) Aspirate the rinse solution, taking care not to damage the monolayers, and collect the solution in a disinfectant-containing trap. **Do not let cells dry.**
- D) Add 1.0-mL of R-Mix™ **ReadyCells**® Refeed Medium to each shell-vial, and proceed with inoculation procedure.

For all other products: (note #2)

- Z) Add 1.0-mL of the appropriate Refeed Medium to each shell-vial.

C. Inoculation Procedure

1. Add 0.2 to 0.4-mL of prepared specimen or control to an appropriately labeled shell-vial.
2. Centrifuge inoculated shell-vials at 700xg for 60-minutes.
3. Remove shell-vials from the centrifuge and place in a 35° to 37°C incubator.
4. Incubate the shell-vials for the laboratory's established period of time (24- to 72-hours).
5. Determine the presence or absence of infectious agents with the appropriate monoclonal antibody staining kit/procedure.

X. Results

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

Warranty Statement

These products are warranted to perform as described in their labeling and the Diagnostic Hybrids, Inc. 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.

ReadyCells, R-Mix, R-Mix Too and Super E-Mix are trademarks or registered trademarks of Diagnostic Hybrids, Inc., in the United States and other countries.

Table 1: ReadyCells® and their virus susceptibility profiles (*Annex II, List B associate products for Chlamydia and cytomegalovirus (CMV) specific.)	
CELL TYPE	INFECTIOUS AGENTS
Hs27* (human foreskin fibroblast)	Intended use: For the cultivation and detection of adenovirus, CMV (cytomegalovirus), echovirus, Herpes simplex (HSV), mumps, poliovirus, rhinovirus, Varicella-zoster (VZV).
McCoy* (mouse fibroblast)	Intended use: For the cultivation and detection of <i>Chlamydia</i> and HSV.
Super E-Mix™: BGMK with hDAF and A549 Buffalo green monkey kidney (derived from African green monkey) with Decay Accelerating Factor and human lung carcinoma	Intended use: For the cultivation and detection of enteroviruses from patient specimens, especially coxsackievirus A, coxsackievirus B, echovirus, and poliovirus. Virus susceptibility profiles of each individual cell type in Super E-Mix: HSV, coxsackievirus B, coxsackievirus A, echovirus, and poliovirus and Adenovirus, HSV, influenza, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.
R-Mix™: Mv1Lu and A549 mink lung and human lung carcinoma	Intended use: For the cultivation and detection of respiratory viruses from patient specimens, especially influenza virus types A and B, adenovirus, respiratory syncytial virus (RSV), metapneumovirus (MPV) and parainfluenza virus types 1, 2, and 3. Virus susceptibility profiles of each individual cell type in R-Mix: HSV, CMV, influenza A and influenza B and Adenovirus, HSV, influenza, MPV, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.
R-Mix Too™: MDCK and A549 Madin-Darby canine kidney and human lung carcinoma	Intended use: For the cultivation and detection of respiratory viruses from patient specimens, especially influenza virus types A and B, adenovirus, RSV, MPV and parainfluenza virus types 1, 2, and 3. Virus susceptibility profiles of each individual cell type in R-Mix Too: influenza A, influenza B, some types of adenovirus, reoviruses, coxsackievirus and Adenovirus, HSV, influenza, MPV, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV.

Table 2: ReadyCells® part numbers	
F-54-0102-24*	ReadyCells ® McCoy frozen cell culture (24 shell-vial box)
F-87-0102-24*	ReadyCells ® Hs27 frozen cell culture (24 shell-vial box)
F-92-0102-24	ReadyCells ® Super E-Mix™ frozen cell culture (24 shell-vial box)
F-96-0102-24	ReadyCells ® R-Mix™ frozen cell culture (24 shell-vial box)
F-97-0102-24	ReadyCells ® R-Mix Too™ frozen cell culture (24 shell-vial box)
99-610-009 (110 volt)	ReadyCells ® Dry Heat Block, 110 volt
99-610-024 (230 volt)	ReadyCells ® Dry Heat Block, 230 volt
05-360075	R-Mix™ Rinse Buffer (ReadyCells ®)
05-370100	R-Mix™ Refeed Medium (ReadyCells ®)
10-320100	RM-02 Refeed Medium (Standard 2% FBS Refeed Medium)
10-340100*	<i>Chlamydia</i> Isolation Medium
10-380100	Super E-Mix™ Refeed Medium