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QUIDEL

FreshCells™ Singles and Mixed

To aid in the diagnosis of diseases associated with infectious agents.

FOR *IN VITRO* DIAGNOSTIC USE

INTENDED USE

FreshCells cultures are intended for virus and/or *Chlamydia* isolation and to aid in the diagnosis of diseases associated with infectious agents. These cells should not be used for serial cell propagation. FreshCells and MixedCells™ are provided in the following formats:

- Singles – monolayers of a single cell line in glass tubes, multi-well plates and shell-vials with or without coverslip. (Table 1)
- Mixed (Patented) – monolayers of two cell lines mixed at approximately equal cell density in multi-well plates and shell-vials with or without coverslip. (Table 2)

SUMMARY

Cell cultures provide the necessary living host systems for the cultivation of viruses and *Chlamydia*. Such cultures are used in the isolation, detection and identification of these infectious agents. The procedure typically involves incubating the specimen with an appropriate cell line. This incubation period is variable and dependent upon the detection system used. The classic detection method is the observation of cellular changes due to the infection of the cells, termed cytopathic effect (CPE). The use of monoclonal antibodies against antigens specific to an infectious agent to confirm the agent's identity 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.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- Cultured cells must be inoculated prior to or on the labeled expiration date.
- As with all methods for virus detection and identification using cultured cells, personnel must be properly trained in virus culture and safe handling techniques as described by the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, 2009 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.
- 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 CDC website listing of the BSL-3 infectious agents and the CDC recommendations at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

- Some FreshCells have been shown to be non-permissive to infection and replication of the etiological agent of Severe Acute Respiratory Syndrome (SARS). For additional information, refer to the CDC website at http://www.cdc.gov/sars/lab/biosafety.html.
- Cultures and specimens should be autoclaved or disinfected with a solution of sodium hypochlorite (1:10 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.

STORAGE

As with any cell culture, FreshCells must be protected from extremes in temperature and light.

Upon receipt, cell cultures should be screened for confluency and stability. During shipment some retraction of the cells may occur. Monolayers less than 60% confluent may need to be placed in a 35°C to 37°C incubator overnight and reassessed the next day.

Once the desired confluency is reached, cell monolayers may be inoculated at that time.

If cells are **not to be inoculated**, the recommended storage is 22°C to 28°C in a clean, dark or low light intensity area and in such a fashion that monolayers remain covered by cell culture medium. This action will not affect cell function or viability.

If desired, uninoculated cells can be stored at 35°C to 37°C in an incubator. **This is not suggested for rapidly growing cells or carcinoma cell lines.** Due to the tendency for these cell line types to overgrowth, which may result in piling or peeling of the monolayers, an incubator temperature **greater than 33°C is not suggested**. (CLSI M41-A, 5.3.2 Maintenance)

Uninoculated MixedCells should be stored at 22°C to 28°C (due to the dissimilar growth rates of the two cell lines) in a clean, dark or low light intensity area and in such a fashion that the monolayers remain covered by cell culture medium.

STABILITY

- 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).

PROCEDURE

Suggested Tube Protocol

1. Prior to inoculation, examine the monolayers for proper morphology. Incubate cell cultures for 2 to 16 hours at 35°C to 37°C.
   NOTE: This step should be considered separately from any prior incubation to improve monolayer confluency.

2. Remove the shipping medium by gentle aspiration being careful not to touch the aspirator tip to the monolayer. **Be sure to remove all of the shipping medium.** This is important for respiratory virus cultures as the shipping media contains FBS which can be potentially inhibitory to influenza virus isolation.
3. Add 1.0 to 2.0 mL of the appropriate Refeed Medium (see Table 3) to each tube.
4. Transfer 0.2 to 0.4 mL of clinical specimen to each tube.
5. Incubate at 35°C to 37°C in a tube rotation drum at 1 to 3 rpm or in a stationary rack as appropriate for the agent suspected.
6. Read for characteristic cytopathic effect (CPE), and/or
7. Fix and stain according to Quidel protocol or, alternatively, the diagnostic manufacturer's monoclonal antibody staining protocol.
8. Examine the monolayer for fluorescent cells using a fluorescence microscope according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.

Suggested Shell-vial Protocol

1. Prior to inoculation, examine the monolayers for proper morphology. Incubate cell cultures for 2 to 16 hours at 35°C to 37°C.
   NOTE: This step should be considered separately from any prior incubation to improve monolayer confluency.
2. Remove the shipping medium by gentle aspiration being careful not to touch the aspirator tip to the monolayer. **Be sure to remove all of the shipping medium.** This is important for respiratory virus cultures as the shipping media contains FBS which can be potentially inhibitory to influenza virus isolation.
3. Add 0.5 to 1.0 mL of the appropriate Refeed Medium (see Table 3) to each shell-vial.
4. Transfer 0.2 to 0.4 mL of clinical specimen to each shell-vial.
5. Centrifuge the inoculated cultures at 700xg for 60 minutes at ambient temperature.
6. Incubate at 35°C to 37°C for 16 to 24 hours, 40 to 48 hours, or longer as appropriate for the agent suspected, or according to the laboratory's established protocol. Additional incubation may be necessary to produce visible CPE prior to staining.
7. Fix and stain according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.
8. Remove the coverslip using a bent teasing needle (commonly used to make tease preps in a Mycology Laboratory). Place the coverslip cell-side down over a small drop of mounting medium on a glass slide.
9. Examine the monolayer for fluorescent cells using a fluorescence microscope according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.

Suggested Multi-well Plate Protocol

1. Prior to inoculation, examine the monolayers for proper morphology. Incubate cell cultures for 2 to 16 hours at 35°C to 37°C.
   NOTE: This step should be considered separately from any prior incubation to improve monolayer confluency.
2. Remove the shipping medium by gentle aspiration being careful not to touch the aspirator tip to the monolayer. **Be sure to remove all of the shipping medium.** This is important for respiratory virus cultures as the shipping media contains FBS which can be potentially inhibitory to influenza virus isolation.
3. Add 0.5 to 1.0 mL of the appropriate Refeed Medium (see Table 3) to each well.
4. Transfer 0.2 to 0.4 mL of clinical specimen to each well.
5. Centrifuge the inoculated cultures at 700xg for 60 minutes at ambient temperature.
6. Place each plate into a 35°C to 37°C incubator with a humidified (85 to 95% to prevent drying), 5% CO_2 (± 1.0% recommended) environment for 16 to 24 hours, 40 to 48 hours, or longer as appropriate for the agent suspected, or according to the laboratory's established protocol. Additional incubation may be necessary to produce visible CPE prior to staining.

7. Fix and stain according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.
   IMPORTANT NOTE: TO PREVENT CRAZING OR CLOUDING OF THE PLASTIC, MONOLAYERS MUST BE FIXED WITH 80% ACETONE / WATER (v/v) FOR NO LONGER THAN 10 MINUTES.
8. Add 2 to 4 drops of mounting medium to each well (sufficient mounting medium to cover the monolayer). Examine each inoculated monolayer for fluorescent cells using a fluorescence microscope.
   - For detection with a standard fluorescence microscope, place a transparent adhesive over the wells, invert the plate and examine the monolayers at 100-400X final magnification.
   - When using an inverted fluorescence microscope, examine the plate directly (non-inverted, without the transparent adhesive).

RESULTS

Refer to diagnostic staining reagent manufacturer's protocol or appropriate reference material for expected results and reporting suggestions.

QUALITY CONTROL

Negative cell controls should be run with each batch of specimens tested for virus or *Chlamydia*. Negative controls consist of uninoculated monolayers that otherwise are handled the same as inoculated monolayers.

Positive virus controls may be run using previously identified viral agents that will produce the result desired from a positive patient sample. While not generally required by regulatory organizations, these may be useful for troubleshooting purposes or for the production of additional external staining controls.

LIMITATIONS

- Aging of cell cultures can result in the loss of sensitivity to virus production. To minimize this effect, FreshCells cultures are available twice a week.
- Harsh conditions encountered during shipment may affect the shelf life of cell cultures. All cultures should be examined for appearance and morphology prior to inoculation.

Table 1. FreshCells Singles and Their Virus Susceptibility Profiles

REF	Cell Line/Origin	Infectious Agents
56-	A549 human lung carcinoma	adenovirus, HSV, influenza, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV, metapneumovirus (MPV)
53-	BGMK Buffalo green monkey kidney	*Chlamydia*, HSV, coxsackie B, poliovirus.
52-	CV-1 African green monkey kidney (not CE marked)	HSV, measles, mumps, rotavirus, VZV
72-	HeLa human cervix adenocarcinoma (not CE marked)	adenovirus, CMV, echovirus, HSV, poliovirus, rhinovirus, vesicular stomatitis (Indiana Strain) virus and VZV
75-	HeLa 229 human cervix adenocarcinoma (not CE marked)	adenovirus, CMV, echovirus, HSV, poliovirus, rhinovirus, vesicular stomatitis (Indiana Strain) virus and VZV
43-	HEL human embryonic lung	adenovirus, CMV, echovirus, HSV, poliovirus, rhinovirus, vesicular stomatitis (Indiana Strain) virus and VZV
57-	HEp-2	adenovirus, coxsackie B, HSV, measles, parainfluenza,

REF	Cell Line/Origin	Infectious Agents
	human epidermoid carcinoma	poliovirus, RSV
86-	LLC-MK2 Original Rhesus monkey kidney	poliovirus type 1, enterovirus, rhinovirus, myxovirus and poxvirus groups
44-	MRHF (HFF) human foreskin fibroblast	adenovirus, CMV, echovirus, HSV, mumps, poliovirus, rhinovirus, VZV
58-	Mv1Lu mink lung	HSV, CMV, influenza A, influenza B
54-	McCoy mouse fibroblast	*Chlamydia*, HSV
83-	MDCK Madin-Darby canine kidney	influenza A, influenza B, some types of adenovirus, reoviruses, coxsackie virus
62-	MNA mouse neuroblastoma	Rabies
51-	MRC-5 human fetal lung	CMV, HSV, adenovirus, influenza, mumps, echovirus, poliovirus, rhinovirus, RSV, VZV
59-	NCI-H292 human, pulmonary muco-epidermoid carcinoma (not CE marked)	vaccinia virus, HSV, adenovirus, measles virus, reoviruses, BK polyomavirus, RSV, some strains of influenza A, most enteroviruses and rhinoviruses
76-	RD human rhabdomyosarcoma	adenovirus, echovirus, HSV, poliovirus
67-	Vero 76 African green monkey kidney cells	adenovirus, coxsackie B, HSV, measles, mumps, poliovirus type 3, rotavirus, rubella, West Nile virus
84-	Vero African green monkey kidney cells	adenovirus, coxsackie B, HSV, measles, mumps, poliovirus type 3, rotavirus, rubella
85-	WI-38 human lung	adenovirus, CMV, echovirus, HSV, mumps, influenza, poliovirus, rhinovirus, RSV, VZV
Additional cell types or formats may be available on request.		

Table 2. MixedCells Mixed Cells and Their Virus Susceptibility Profiles

REF	Cell Line/Origin	Infectious Agents
92-	**Super E-Mix™**: sBGMK and A549 sBGMK/Buffalo green monkey kidney with Degradation Accelerating Factor and A549/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 A549/human lung carcinoma	influenza A, influenza B, HSV, CMV and adenovirus, HSV, influenza, MPV, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV
97-	**R-Mix Too™**: MDCK and A549 Madin-Darby canine kidney and A549/human lung carcinoma	influenza A, influenza B, and adenovirus, HSV, influenza, MPV, measles, mumps,

REF	Cell Line/Origin	Infectious Agents
		parainfluenza, poliovirus, RSV, rotavirus, VZV
98-	**H&V-Mix™***: CV-1 and MRC-5 CV-1/African green monkey kidney and MRC-5/human fetal lung	 HSV, VZV, poliovirus type 1, some encephalitis viruses, SV40 virus and adenovirus, CMV, echovirus, HSV, influenza, mumps, poliovirus, rhinovirus, RSV, VZV

Table 3. Cell Culture Medium

REF # (100 mL and 500 mL)		**Description**
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
10-340100		Chlamydia Isolation 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
10-160100	10-160500	Zero-Serum Refeed Medium - MEM
10-170100	10-170500	Refeed Medium 5% FBS

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.

REFERENCES

1. *Viral Culture; Approved Guideline M41-A*. Vol. 26, No. 7. Clinical and Laboratory Standards Institute, Wayne, PA. 2006.
2. CDC-NIH manual. *Biosafety in Microbiological and Biomedical Laboratories (BMBL),* 5th edition, 2009, [http://www.cdc.gov/biosafety/publications/bmbl5/index.htm]

REF 055 – FreshCells Singles and Mixed

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EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Diagnostic Hybrids, Inc. – a subsidiary of Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
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