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Practical Studies in Vero Cell and Human Amnion Cells for Viral and Stem Cell Applications

Ahmed Mahfouz*

Abstract

Vero cells are one of the most popular mammalian continuous cells in molecular, cellular, and microbiological research. African green monkey kidneys are used to make these. One continuous cell line is the Vero cell line. This cell line is dependent on anchorage. Vero cells can be cultured in suspension because they are anchorage independent. Thought to be non-tumorigenic cells that came from a female Chlorocebus sabaeus Numerous viruses can infect vero cells. Its lack of interferon expression is thought to be one of the causes of this. When viruses infect cells, the signal peptide interferon is not produced, impairing the cell's ability to fight off infection. The second type of cell we focus on is the human amnion cell, which is an embryonic stem cell that can grow fast. This stem cell can be targeted in regenerative medicine to treat colon cancer and other tumor types. We suppose that scientists can use Hu-amnion cells in drug discovery and gene editing by induced stem cells to form scaffold structure and tissue printing. Also, Hu-amnion cells can be seeded to be used in vaccines by vector-mediated vaccines or small subunit vaccines. Our research focuses on the separation of cells from different parts using this cell in the application of stem cells, viral replication, and vaccine production.

Keywords: Vero cells, human amnion cells, stem cell therapy, viral replication, vaccine production

INTRODUCTION

Types

Commercially accessible vero cell lines include vero, vero 76, and vero E6. Cell lines CRL-81 (vero), CRL-1286 (Vero C1008), and CRL-1587 (vero 76) from ATCC are frequently used. ECACC cell lines 85020206 (vero C1008), 88020401 (vero-WHO), are also used. 85020205 (vero 76) and 84113001 (vero) often used [1].

Uses

Vero has been widely employed in virology research, but it has also been used in numerous other applications, such as the study and propagation of intracellular parasites (such as *Neospora*) and bacteria (such as *Rickettsia spp*.; UNIT 3A.4), as well as the molecular-level assessment of how chemicals, poisons, and other materials affect mammalian cells. Additionally, research is being done on Vero cells as a cell substrate to produce certain oncolytic viruses.

In the United States, Vero cells have been approved for the manufacture of both live (smallpox, rotavirus) and novel vaccines [2].

Isolation and Growth

Propagation, maintenance and preparation of vero cell culture has been depicted in Table 1.

***Author for Correspondence**
Ahmed Mahfouz
E-mail: ags11@fayoum.edu.eg

Microbiologist, Virologist Faculty of Science, Microbiology and Botany Department Fayoum University, Egypt

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Table 1. Propagation, maintenance and preparation of vero cell culture.

Basic Protocol 1	**Basic Protocol 2**	**Basic Protocol 3**
Vero Cell Culture Propagation from Frozen Stocks This protocol explains how to start a Vero cell culture from frozen stock. When a frozen store can be restored	*Maintenance of Vero Cell Culture* This protocol describes a general method for the subculturing of Vero cells in 75cm2 tissue culture flasks.	*Preparation of Frozen Stocks of Vero Cell Culture*
Materials Materials Vero cell stock, either at –80°C or frozen under liquid nitrogen 10% heat-inactivated fetal bovine serum (FBS) added to Dulbecco's modified Eagle medium (DMEM) and filter sterilized (according to the recipe) 15 mL conical tubes, sterile tissue culture flasks measuring 25 or 50 cm^2 and having vented tops, sterile serological pipets, and sterile 70% ethanol solution (used to clean the laminar flow hood and items carried inside it).	*Materials* Materials Vero cells cultured to a confluent monolayer in a 75 cm^2 flask with a vented cover in filter-sterilized DPBS devoid of calcium and magnesium (refer to APPENDIX 2A) 1X filter-sterilized trypsin-EDTA in DPBS without calcium or magnesium (refer to the recipe) 10% heat-inactivated. After filter sterilization by adding FBS to DMEM, a sterile 15 mL conical tube, sterile serological pipette. and a 75 cm^2 sterile tissue culture flask with a vented cap was used (see recipe).	*Materials* Materials Vero cells cultured to a confluent monolayer in a 75 cm^2 flask with a vented cover in filter-sterilized DPBS devoid of calcium and magnesium (refer to APPENDIX 2A) 1X filter-sterilized trypsin-EDTA in DPBS without calcium or magnesium (refer to the recipe) 20% heat-inactivated FBS added to DMEM and filter-sterilized (according to the formula) DMSO cryovials that may be Sterile 15 mL conical tube. Sterile serological pipette and frozen at -80°C or liquid nitrogen.
Thaw the Vero cell vial (cryovial) as quickly as possible by gently whirling it in a water bath at 37°C. Water in the bath is one source that can cause cell contamination. Keep the cryovial's O-ring and cap out of the water to lower the chance of contamination. Bathing water is one source that can cause cellular contamination. Place the Vero cell suspension in a 15 mL conical tube with 10 mL of DMEM supplemented with FBS after transferring it from the cryovial. The cryopreservant dimethyl sulfoxide (DMSO), which is present in frozen cell supplies, may be detrimental to the cells. For this reason, cells should be diluted and fixed in DMSO before being transferred to tissue culture flasks after thawing. In addition to DMEM, the medium allowed cells to be resuspended in 5–10 mL of DMEM, with the supernatant removed and discarded. Supplemented with 10% FBS. Vero cells that are started in a small tissue culture flask (25 cm^2 or 50 cm^2) recover better after freezing. Resuspend	Take the growing media out of the Vero cells' confluent monolayer. Use 10mL 1X DPBS to wash the cells. Trypsin inhibitors are present in serum, hence it is critical to clean any leftover media with DPBS. After adding 5 mL of 1X trypsin-EDTA, incubate the cells for two to three minutes at 37°C, or until they begin to streak as they separate from the flask. Cells may separate if the flask is gently shaken or tapped. To neutralize trypsin-EDTA, 5 mL of DMEM containing 10% FBS was added. Use a pipette to carefully break up any cell clumps as you wash the cells in the medium. Remove the cell suspension from the vial and transfer it to a sterile 15 mL conical tube. Centrifuge at an ambient temperature for 5 minutes at 200 × g.	To reach a final concentration of 10% DMSO, mix 1 mL DMSO with 9 mL of the supplemented DMEM in a 15 mL conical tube in a laminar flow hood. After FBS has been added to the DMEM, DMSO should be added since it will break down cellulose acetate membranes, which are frequently used for filter-sterilization. Nylon membrane filters can be used to filter the DMSO; alternatively, the container should only be opened in a sterile laminar flow hood. Take the growing media out of the Vero cells' confluent monolayer. Use 10 mL 1X DPBS to wash the cells. Trypsin inhibitors are present in serum, hence it is critical to clean any leftover media with DPBS. After adding 5 mL of 1X trypsin-EDTA, incubate the cells for two to three minutes at 37°C, or until they begin to streak as they separate from the flask. Cells may separate if the flask is gently shaken or tapped. To render the trypsin-EDTA inactive, add 5 mL of DMEM containing 20% FBS. Use a pipette to carefully break up any cell clumps as you wash the cells in the

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the cells in 5 mL of medium if you are using a 25 cm2 flask, and 10 mL of media if you are using a 50 cm^2 flask. Fill a tissue culture flask with a vented cover with the Vero cell suspension. Incubate the vials at 37°C with 5% CO_2. Keep an eye on cells every day or every other day. Flip the media every three to four days. Pass cells into fresh tissue culture flasks once they form a confluent monolayer of greater than 90% (according to Basic Protocol 2). It may take a week or longer for vero cells to be prepared for passage since they recover slowly after freezing. Before the Vero cells attain their typical growth rate, two or three passes can be required.	Re-suspend cells in 10mL DMEM containing 10% FBS after removing and discarding the supernatant. In 75cm2 Prepare the desired cell dilution in 12–20 mL DMEM with 10% FBS in a cell culture flask with a vented cap. Typical dilutions for ordinary cell culture range from 1:5 to 1:10. F	medium. Remove the cell suspension from the vial and transfer it to a sterile 15 mL conical tube. Centrifuge an ambient temperature for 5 minutes at 200 × g. After removing and discarding the supernatant, resuspend the cells in 10 milliliters of DMEM containing 10% DMSO and 20% FBS. Respectively during snowmelt, FBS and DMSO were used to preserve the cells. Each cryovial should contain 1 mL of resuspended cells. Cells should be gradually frozen to -80°C before being stored there or, if preferred, in liquid nitrogen. The cells should ideally be frozen when the temperature drops by –1°C every minute. This can be done using a cryogenic container, such as the Nalgene Cryo 1°C cryogenic container. Another option is to place the cells at 4°C for a few hours, then -20°C for the night, then –80°C for the night, and finally either remain at –80°C or move them into liquid nitrogen storage.

Method 4
Serum Containing Medium

Cell adaptation to serum-free media following normal technique, 20X106 Vero cells were cryopreserved in MEM þ 20% FCS þ 10% DMSO in an ampoule. The cells were then revived in serum-containing media (SC: MEM þ 10% FCS) [3].

Cells were left to adhere for three hours at 37°C in an incubator with 5% CO_2. The cells were then incubated at 37°C with 5% CO_2 after the medium was changed to the one to be investigated. MEM for culture media and chemicals (Cat. No. 61100-087) and M199 (Cat. No. 31100027).

Method 5

Growth of cells in a static culture according to Trabelsi's instructions, vero cells were cultivated in SC media (MEM þ 10% FCS). Similar conditions were used for cell static cultures in serum or animal-component free medium: a seeding density of 8 104 cells cm 2, 37C, and 5% CO_2. Cell culture was carried out in duplicate either in 24 well cell culture plates or in 25 cm^2 cell culture flasks (corning, acton, MA). The working volume for the studies carried out in 24 well plates and T-flasks was 1.5 ml and 10 ml, respectively [4–6].

Method 6

Vero E6 and vero CCL-81 cells were cultivated in Dulbecco's minimum essential medium, which was enhanced with penicillin-streptomycin (10,000 U/ml), 10% heat-inactivated fetal bovine serum, and HEPES [7].

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Growth Using Micro Carriers

Microcarrier (MC) preparation in accordance with the manufacturer's instructions, Hillex®, plustic plus, cytodex 1, and cytodex 3 micro-carriers were manufactured and sterilized. When the cytodex 1 and cytodex 3 microcarriers contain 3 g/l each. were swollen twice with Ca_2^+, Mg_2^+-free PBS and autoclaved at 115°C for 15 min, Hillex® (14 g/L) and plustic plus (20 g/L). with a microcarrier. Prior to usage, all microcarriers were cultured with RPMI medium.

After discarding the cell dissociation media, the cells were twice rinsed with phosphate buffer saline (PBS), filled with 2 ml of the enzyme accutase, and incubated for 2 minutes at 37°C in a 75 cm³ flask. After that, the dislodged cells were combined with RPMI medium that contained 10% serum and put into inoculum flasks.

A 250 ml spinner flask containing 200 ml of cultured cells (Bellco Biotechnology, U.S.A.) was used and the cultures were conducted at 37°C in an incubator with 5% CO_2. When the media's initial pH was set to 7, the stirring speed was kept at 30 rpm. Two × 105 cells/ml were used to inoculate the spinners. Sampling was done every eight hours during the 96-hour period.

Culture in a bioreactor the cultures were carried out in a two-liter bioreactor (Labfors 3, Switzerland) with a marine impeller and a one-liter working volume. in a bioreactor 900 mL of RPMI medium and 3 g/L Cytodex 3 were mixed, and the mixture was incubated for three hours to start the microcarrier culture. Using PBS and the enzyme accutase, vero cells were separated from T-flasks, and 10% inoculums were introduced to the bioreactor culture. 2.5 × 105 cells/ml were used to seed the culture, which was then constantly shaken at 70 rpm. The following parameters were used throughout the cell culture proliferation step: a pH of 7.2, a temperature of 37°C, an agitation rate of 70 rpm, and a pO2 maintained at 30% air saturation by introducing air or pure oxygen as needed (Figure 1).

Sampling occurred every eight hours throughout the 96-hour period of testing [8].

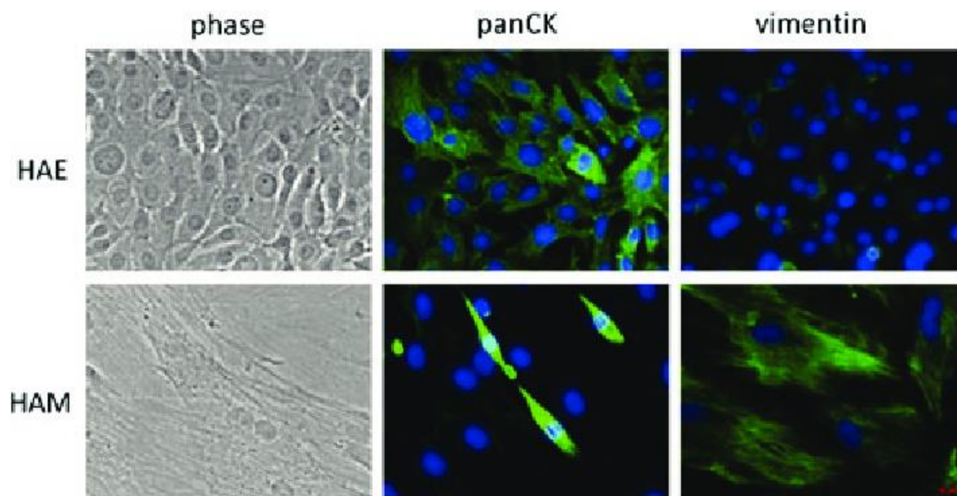

Figure 1. Morphology of HAE and HAM cells. HAE and HAM cells are stained with anti-pan-CK or vimentin antibodies, respectively (green). Nuclear staining was performed with Hoechst (blue).

Amnion Cells

In the realm of regenerative medicine, human amniotic epithelial cells (hAECs) produced from placental tissues have drawn a lot of interest.

Although amniotic cells have been demonstrated to have the capacity for multi-differentiation, their differentiation efficiency is poor. Amnion-derived cells are a diverse cell population that include stem cells, while having stem cell traits and the ability to differentiate into other cell types. In terms of

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morphology, cultivated HAM cells are spindle-shaped, while cultured HAE cells are tiny and spherical cells [9].

It is well established that HAESCs have paracrine, immune-privilege, and stem-cell-like plasticity. Furthermore, because to their non-tumorigenicity and the fact that they are often thrown away after parturition, they do not raise ethical concerns.

Cell Separation Process

The amniotic membrane is manually removed from the placental bundle with consent after a straightforward cesarean section.

Growth Media

Method 1

Tissues were removed and treated with trypsin (2 mg/mL) at 37 °C for 20 min to lyse HAE cells. The epithelial cells were eliminated after this procedure was repeated many times. Tissue sections were cultured in Dulbecco's modified eagle's medium at 37°C for 60 min to isolate HAM cells. (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with collagenase (0.75 mg/mL) and DNase (0.075 mg/mL). By filtering the mixture via gauze and centrifuging, the scattered HAE or HAM cells were gathered [10].

Method 2

According to Sato JD.et al [10], method without serum for isolating and cultivating hAESC. The culture media used for hAESCs is F12/DMEM, 10% KSR (KnockOut serum replacement), 2 mmol/L L-glutamine, 1% nonessential amino acids, 55 µmol/L 2-mer-captoethanol, 1 mmol/L sodium pyruvate, 1% antibiotic–antimycotic, and 10 ng/mL EGF, to put it briefly.

Even after cryopreservation, hAESCs exhibit improved growth behavior and consistent morphology in the serum-free system. Furthermore, in the serum-free state, hAESCs retain their characteristics including pluripotency, limited immunogenicity, and modest immunoregulatory activity.

Method 3

Using CRC technology to expand hAECs where 10% fetal bovine serum (FBS) was added to CRC medium (CM) F medium conditioned by J2 cells for three days. Filtered to remove debris and dead J2 cells, then combined with fresh F medium in a 3:1 (volume/volume) ratio, resulting in spontaneous and regenerative CM. nutrient mixture F-12 (HAM)-DMEM (Invitrogen), 5% human serum (HS) (GIBCO) or 10% FBS (GIBCO), hydrocortisone 0.4 µg/mL (sigma), insulin 5 mcg/mL (Sigma); 8.4 ng/ml cholera toxi sigma), 10 ng/ml EGF (invitrogen), 24 µg/ml adenine (sigma), and 5–10 µM Y-27632 (enzo health sciences) made up the fresh F medium (Figure 2). Add additional new 5uM Y-27632 to media F before use. Each cell line was stored at 37°C in an incubator humidified with 5% CO2. CRC cryopreservation medium was used for cryopreservation of uncultured cells and cells from each passage. Contains 90% HS or FBS, 10% DMSO and 5 mM Y-27632 (Tables 2 and 3) [10].

Table 2. Isolation of amniotic epithelial stem cells (AESCs) from varied species.

Species	Stage	Enzymes	Digestion Time	Cellular Yield
Rat	Embryonic day 15 (E15)	0.125% Trypsin	15 min	N. M.
Ovine	90 days of pregnancy	Trypsin	20 min	2–10 × 106
Horse	N. M.	Collagenase type I + 0.25% Trypsin	3 h + 2 min	10 × 106
Chicken	6-day-old chicken embryos	0.05% Trypsin	1 min + 5 min	N.M.
Feline	40–45 days of pregnancy	0.05% Trypsin	40 min	N.M.
Sheep	N.M.	0.25% Trypsin	30 min	N.M.

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Uses

Uses in the field of regenerative medicine. Furthermore, transplanting such cells may be helpful in treating a variety of inflammatory and degenerative disorders due to their capacity to engraft in damaged organs and alter the immunological and repair responses of host tissues [11–14]. Conditions, such as diabetes, systemic lupus erythematosus, autoimmune uveitis, liver fibrosis, wound healing, Hashimoto's thyroiditis, and intrauterine adhesions.

Table 3. Clinical trials utilizing amnion membrane and hAESCs as biological in tervention.

	Category	Registration Number	Disease	Phases	Age	Country
Amnion Membrane	Ophthalmology.	NCT02168790	Ocular surface disorders.	Early 1	18–80 years	Germany.
		NCT01341223	Ocular surface reconstruction.	N/A	18–50 years	China.
	Skin.	NCT03754218	Burn.	Early 1	18–65 years	United States.
		ACTRN12619001050145	Burn.	N/A	20–50 years	Pakistan.
		ACTRN12618001632280	Skin regeneration.	N/A	20–45 years	Pakistan.
		ACTRN12618001631291	Burn.	N/A	10–40 years	Pakistan.
Human Amniotic Epithelial Stem Cells (hAESCs)	Ophthalmology.	NCT00344708	Corneal epithelial dystrophy.	N/A	18–88 years	United States.
	Neurology.	NCT02961712	HTLV-1 associated myelopathy.	1	18–75 years	China.
		NCT03107975	Spastic cerebral palsy.	1	1–5 years	China.
		NCT04414813	Parkinson's disease.	Early 1	30–70 years	China.
		ACTRN12618000076279	Ischemic stroke.	1	18–85 years	Australia.
	Gynecology.	NCT02912104	Primary ovarian insufficiency, Premature ovarian failure.	1	20–39 years	China.
		NCT03223454	Asherman's syndrome.	1	20–40 years	China.
		NCT03207412	Premature ovarian failure.	N/A	18–40 years	China.
		NCT03381807	Intrauterine adhesion.	Early 1	20–45 years	China.
	Pneumology.	NCT02959333	Bronchial fistula.	1	18–75 years	China.
		ACTRN12614000174684	Bronchopulmonary dysplasia.	1	36 weeks	Australia.

CONCLUSIONS

From our study for cell line, such as vero cells, hu-amnion cells we can apply them in viral application, such as vaccination and immunotherapy. Also, the application of stem cell needs to cell line seeding and cultivation by induction cancerous stem cells we can get the continuous cell line, apply this cell line in molecular docking and drug discovery can get new active compounds for diseases.

With over 40 years of combined experience in the vaccine industry, the vero cell line is the most widely used continuous cell line to produce viral vaccines. It has emerged as a crucial screening and discovery tool to aid in the global research and development efforts during the COVID-19 pandemic.

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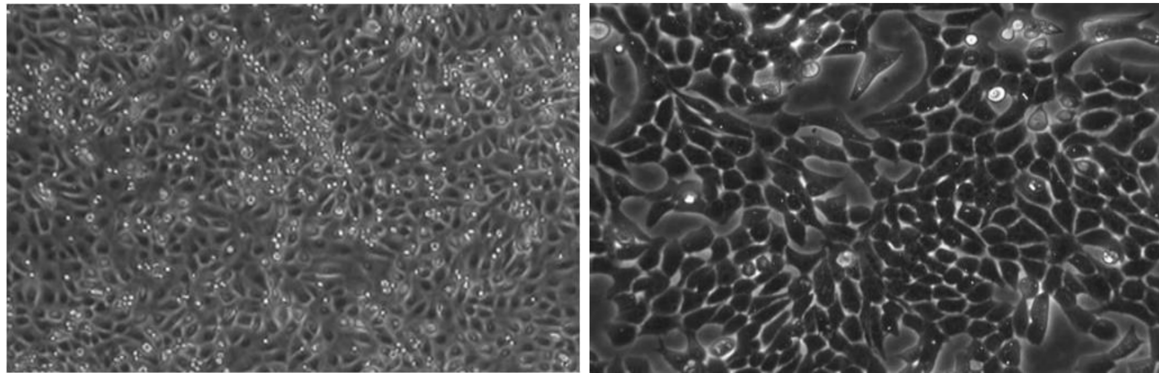
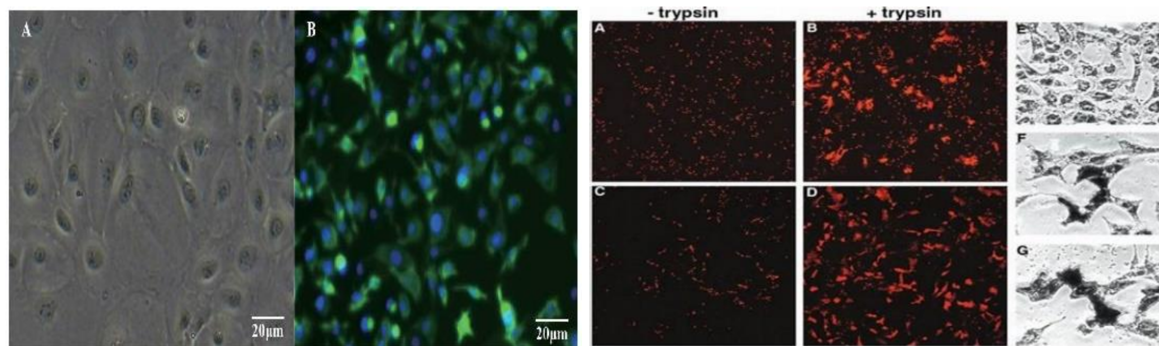


A. Transplantation of vero cell.

B. Verocell Dissociation by trypsin forming monolayer.



C. HESCs size range to 20 nm.

C. HESCs confluency.

Figure 2. Morphology and growth pattern of vero cells and human embryonic stem cells (HESCs). (A) vero cell transplants show differentiated cell morphology. (B) monolayer formation by vero cells after cleavage with trypsin. (C) size distribution of HESCs with cells up to 20 µm showing both phase contrast and fluorescence images. (D) confluence state of HESCs under conditions where difference including with and without trypsin treatment as seen by fluorescence microscopy.

The vero cell portfolio has the potential to evolve into an affordable, high-throughput platform available globally. By using gene editing to increase virus production achieve high-throughput production and strong process scalability). The vero cell portfolio serves as a platform for there is a wide variety of research on the culture of viruses**.** By effectively suppressing the host antiviral response to infection and converting vero cells to suspension culture to allow for more efficient scale-up, several attempts have been made to genetically modify this cell line to get around the restrictions on viral generation yield.

Our knowledge of the phenotypic processes that control vero cells' adaptation to suspension, as well as the host-virus interactions that underline the vero cells' affinity for important emerging pathogens, has been hampered by the absence of a reference genome for the vero cell line. More significantly, this has hindered our ability to use Vero genome editing to redesign high-yield vaccine production processes.

Suggestion

We suggest the application of vero cell and hu-amnion cell in more branches of molecular biology, they can be used in genome editing. By editing genes responsible for receptor formation, we can use them in drug targeting pathways and measuring the in silico cytotoxicity of drug on cell.

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qualification hand protocols. Thanking my academic department of microbiology for providing microbiological safety partition for isolation and seeding of cell, thank my team for the effective and powerful efforts to share help for this research.

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