SERUM FREE CELL CRYOPRESERVATION PRODUCTS

Nomenclature	Cat No.	Use and Description	Specification and storage
Serum Free Cell Cryopreservation Media	NC1001.1	It contains no serum, or rHSA. It has no human-derived or animal-derived components, and is more suitable for clinical study.No programed cooling is required. Double-cryoprotectant formula is used, and the cell recovery rate is above 90%.It supports storage of stem cells and immune cells. It can be used for long-term storage of many types of mammalian cells.	100 mL/vial Store at 2-8°C Expiry date: 12 months
Serum Free Cell Cryopreservation Media	NC1001.2	It is serum free, and contains no animal-derived components such as bovine serum albumin. It is more suitable for scientific research.No programed cooling is required. Double-cryoprotectant formula is used, and the cell recovery rate is above 90%.It supports storage of stem cells and immune cells. It can be usedfor long-term storage of many types of mammalian cells.	100 mL/vial Store at 2-8°C Expiry date: 12 months

MSC CULTURE SET

Cat No.	Nomenclature	Use	Expiry date
NC0103	MSC Serum Free Media	The product contains rHSA, has no human-derived or animal-derived components, is more suitable for clinical study, and is used for primary separation and follow-up subculturing of umbilical cord and AMSCs	500 mL/vial Store at 2-8°C, with the expiry date of 12 months
NC0103.S	MSC medium supplement	This product should be used in conjunction with the MSC serum free media (Cat. No.: NC0103). 500 mL media can be added per 5 mL	5 mL/vial Store at -20°C, with the expiry date of 12 months

NK PURE FACTOR CULTURE SET (PERIPHERAL BLOOD & UMBILICAL CORD BLOOD)

Nomenclature	Specification	Applicable samples	Performance description	Expiry date
2 L system	2 vials of NK media (1L) + kit (2L)	Peripheral blood, concentrated white cells, cryopreserved monocyte	Cell count 4-6 billion, positive rate 60%-75%; no coating is required	12 month
2 L system	2 vials of NK media (1L) + kit (2L)	Umbilical cord blood	Cell count 4-6 billion, positive rate 60%-75%; no coating is required	12 month

CIK PURE FACTOR CULTURE PACKAGE (PERIPHERAL BLOOD & UMBILICAL CORD BLOOD)

Nomenclature	Specification	Applicable samples	Performance description	Expiry date
2 L system	2 vials of immune media (1L) + kit (2L)	Peripheral blood, concentrated white cells, cryopreserved monocyte	Cell count 6-12 billion, positive rate 30%-60%; no coating is required	12 month
2 L system	2 vials of immune media (1L) + kit (2L)	Umbilical cord blood	Cell count 6-12 billion, positive rate 30%-60%; no coating is required	12 month

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YOCON 友康®

SERUM FREE CELL CRYOPRESERVATION MEDIA

GMP

- 1.No bovine serum or animal-derived components. High safety
- 2.Ready-to-use, no need of cryopreservation. It is stable after being stored at 2-8°C
- 3.No programed cooling is required, and the product can be directly injected at -80°C, and placed in liquid nitrogen after 12 hours
- 4.Cell recovery rate above 90%
- 5.It supports high-density cryopreservation of MSC cells, 10 times conventional serum cryopreservation media

Product Overview

Use

1.Storage of stem cells such as hematopoietic stem cells and MSCs.

2.Storage of immune cells such as T lymphocytes and NK cells.

3.Storage of other mammalian cells.

It is an upgraded product of traditional serum cryopreservation media.

This product is especially suitable for study and development enterprises of cell drugs.

This product contains no animal-derived components, has definite components, and significantly reduces the validation workload and difficulty of application of cell drugs.

Product advantages

1.No bovine serum or animal-derived components. High safety.

2.Ready-to-use, no need of cryopreservation. It is stable after being stored at 2-8°C.

3.No programed cooling is required, and the product can be directly injected at -80°C, and placed in liquid nitrogen after 12 hours.

4.Cell recovery rate＞90%.

5.It supports high-density cryopreservation of MSC cells, 10 times conventional serum cryopreservation media.

6.This product is especially suitable for enterprises applying for cell drugs.

Product principle

The Yocon Biology patented formula of cryopreservation media is used, in which the serum is replaced with many protein components including recombinant human albumin (the fifth component of bovine serum albumin, BSA, is used for scientific research products)

The single DMSO protective effect is replaced by composite cryoprotectants including DMSO

The internal protectant in the composite protectant is easy to penetrate the cell membrane and prevent cell injury caused by ice crystals developed by the water molecules inside the cell in the cell cryopreservation process

The external protectant in the composite protectant can competitively bind water molecules on the cell membrane outside the cell membrane before the solution develops ice crystals, thereby reducing the extracellular electrolyte concentration of the solution and reducing the number of cations entering the cell

Under the synergistic protective effects inside and outside of the cell, the damage to the cells caused by rapid temperature decrease and temperature increase will be significantly reduced, thus significantly improving the recovery viability of cells after cryopreservation

Performance comparison

Traditional Cell Cryopreservation Media VS Serum Free Cell Cryopreservation Media

No	Difference	Traditional cell cryopreservation media	Serum Free Cell Cryopreservation Media
1	Temperature storage conditions of the product before use	Cryo-preserve at -20°C and transport with dry ice	Store at 2-8°C, and transport with ice packs
2	Time for thawing cryopreservation media before cell storage	30min	Ready-to-use, no need of waiting
3	Is programed cooling required for cell cryopreservation	4°C, -20°C, -40°C, and -80°C programed cooling is required. Then the product is stored in liquid nitrogen	No programed cooling is required. The product is directly put into -80°C refrigerator. Then the product is stored in liquid nitrogen
4	Can it be used in clinical study?	Not applicable The effect of the residual serum on the cells can be significantly eliminated only after the cells are passaged for more than 3 generations	Applicable The product contains no bovine serum or animal-derived component, and is added with recombinant human albumin

No	Cell line	Traditional cell cryopreservation media	YOCON Serum Free Cell Cryopreservation Media	Recommended cryopreservation density
1	Monocytes (primary separation)	50%	90%	0.1-1.0×10^7cells/mL
2	MSCs	80%	95%	0.1-2.5×10^7 cells/mL
3	NK cells/CIK cells	80%	95%	0.1-1.0×10^7cells/mL
4	MDCK (canine kidney cells)	90%	95%	0.5-3.0×10^6 cells/mL
5	Hybridoma cells	80%	90%	1-3×10^6 cells/mL

Special note

1.The cell recovery data in the "traditional cell cryopreservation media" were obtained by using the programed cooling method in the cell cryopreservation process

2.The cell recovery data in "YOCON serum free cell cryopreservation media" and "Japanese Q serum free cell cryopreservation media" were not obtained by programed cooling in the cell cryopreservation stage. Cells were placed directly in a -80°C freezer and then in liquid nitrogen

3."Recommended cryopreservation density" refers to the recommended cryopreservation density when cells are cryopreserved with YOCON serum free cell cryopreservation media. For the recommended cryopreservation density of other cells, please contact us for consultation

Monocytes (primary separation): comparison of storage, recovery and culture in traditional cryopreservation media and serum free cryopreservation media

Photo of cells stored in the traditional cell cryopreservation media after recovery

Photo of cells stored in the YOCON serum free cryopreservation media at 0 h after recovery

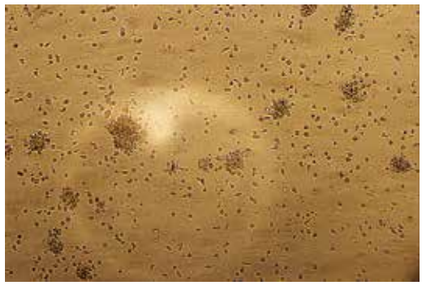


Photo of cells stored in the traditional cell cryopreservation media at 120 h after recovery

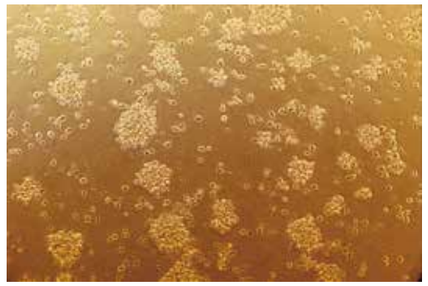


Photo of cells stored in the YOCON serum free cryopreservation media at 72 h after recovery

MSC cells: comparison of storage, recovery and culture in traditional cryopreservation media and serum free cryopreservation media

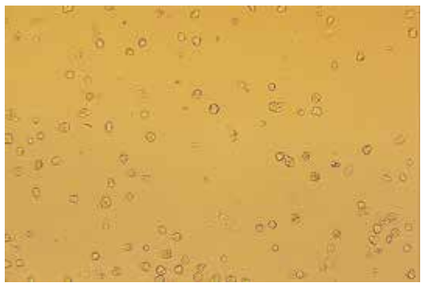


Photo of cells stored in the traditional cell cryopreservation media after recovery

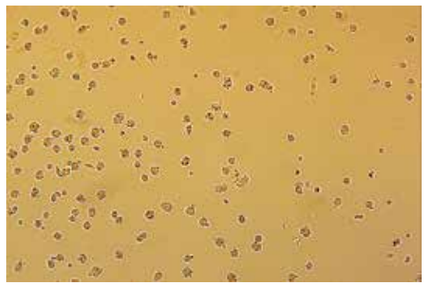


Photo of cells stored in the YOCON serum free cryopreservation media at 0 h after recovery

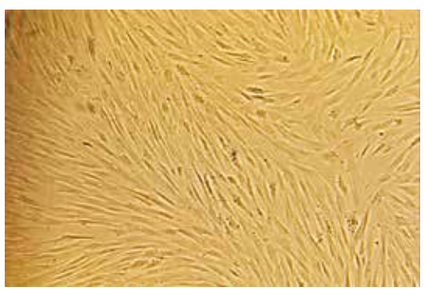


Photo of cells stored in the traditional cell cryopreservation media at 120 h after recovery

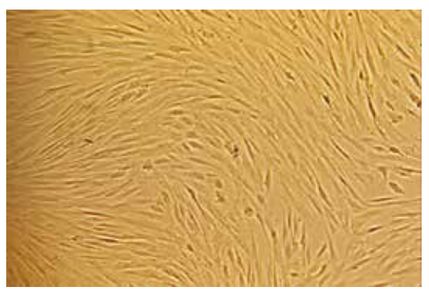


Photo of cells stored in the YOCON serum free cryopreservation media at 72 h after recovery

MDCK cells (canine kidney cells): comparison of storage, recovery and culture in traditional cryopreservation media and serum free cryopreservation media

Photo of cells stored in the traditional cell cryopreservation media after recovery

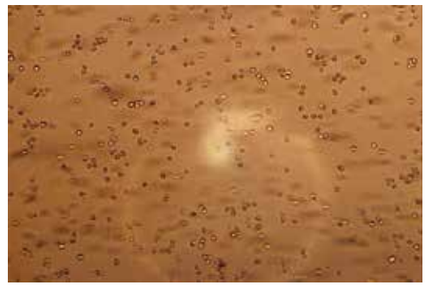


Photo of cells stored in the YOCON serum free cryopreservation media at 0 h after recovery

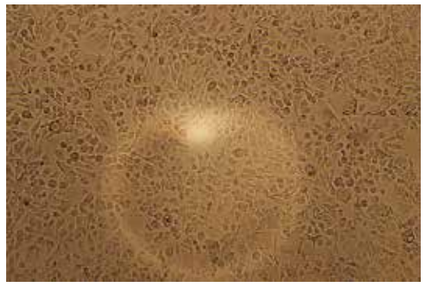


Photo of cells stored in the traditional cell cryopreservation media at 120 h after recovery

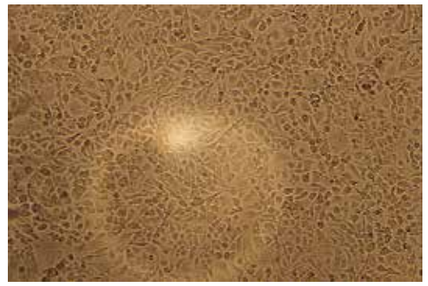


Photo of cells stored in the YOCON serum free cryopreservation media at 48 h after recovery

Hybridoma cells: comparison of storage, recovery and culture in traditional cryopreservation media and serum free cryopreservation media

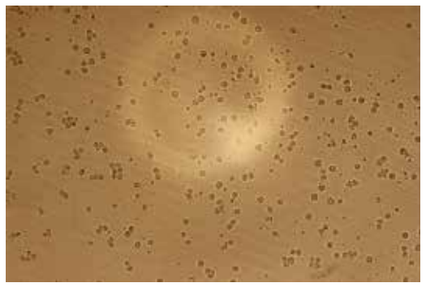


Photo of cells stored in the traditional cell cryopreservation media after recovery

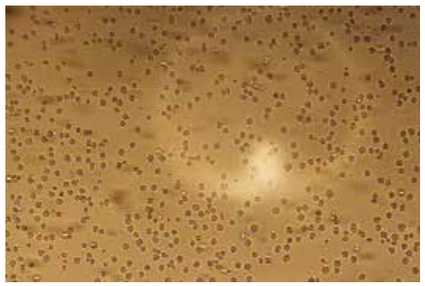


Photo of cells stored in the YOCON serum free cryopreservation media at 0 h after recovery

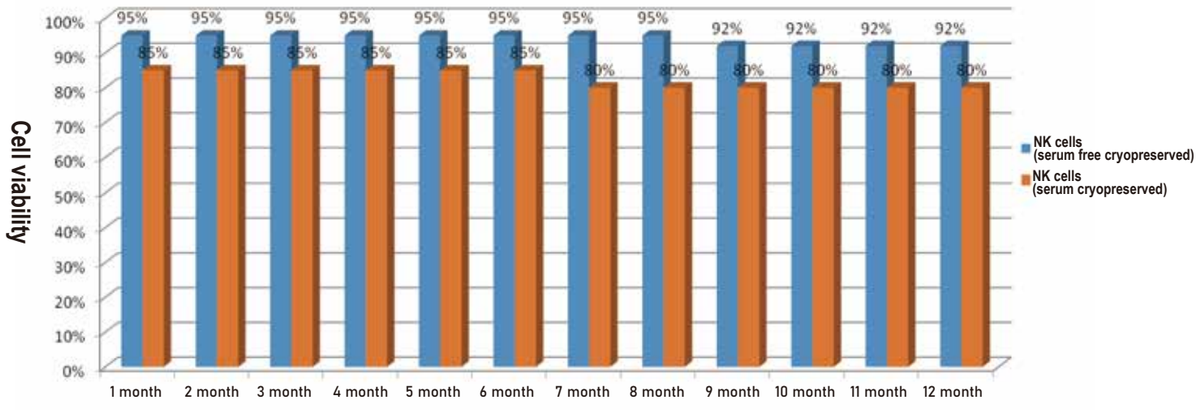
Photo of cells stored in the traditional cell cryopreservation media at 120 h after recovery

Photo of cells stored in the YOCON serum free cryopreservation media at 48 h after recovery

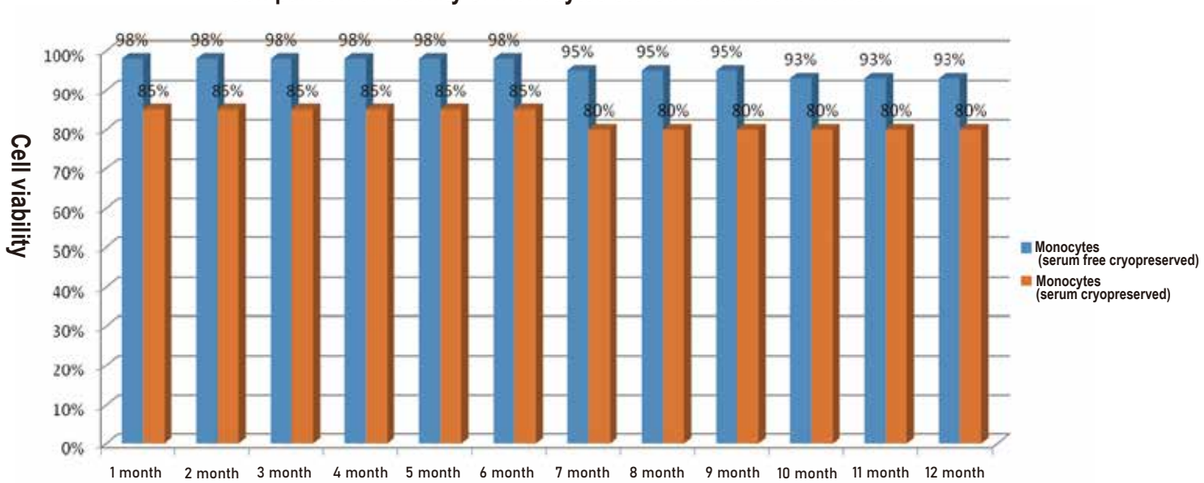
Immune cell cryo-preservation

cells	1 month	2 month	3 month	4 month	5 month	6 month	7 month	8 month	9 month	10 month	11 month	12 month
NK cells (serum free cryopreserved)	95%	95%	95%	95%	95%	95%	95%	95%	92%	92%	92%	92%
NK cells (serum cryopreserved)	85%	85%	85%	85%	85%	85%	80%	80%	80%	80%	80%	80%
CIK cells (serum free cryopreserved)	95%	95%	95%	95%	95%	95%	95%	95%	95%	95%	95%	95%
CIK cells (serum cryopreserved)	85%	85%	85%	85%	85%	85%	80%	80%	80%	80%	80%	80%
Monocytes (serum free cryopreserved)	98%	98%	98%	98%	98%	98%	95%	95%	95%	93%	93%	93%
Monocytes (serum cryopreserved)	85%	85%	85%	85%	85%	85%	80%	80%	80%	80%	80%	80%
T cells (serum free cryopreserved)	98%	98%	98%	98%	98%	98%	95%	95%	95%	95%	95%	95%
T cells (serum cryopreserved)	85%	85%	85%	85%	85%	85%	80%	80%	80%	80%	80%	80%

Comparison of viability of NK cells stored for different months



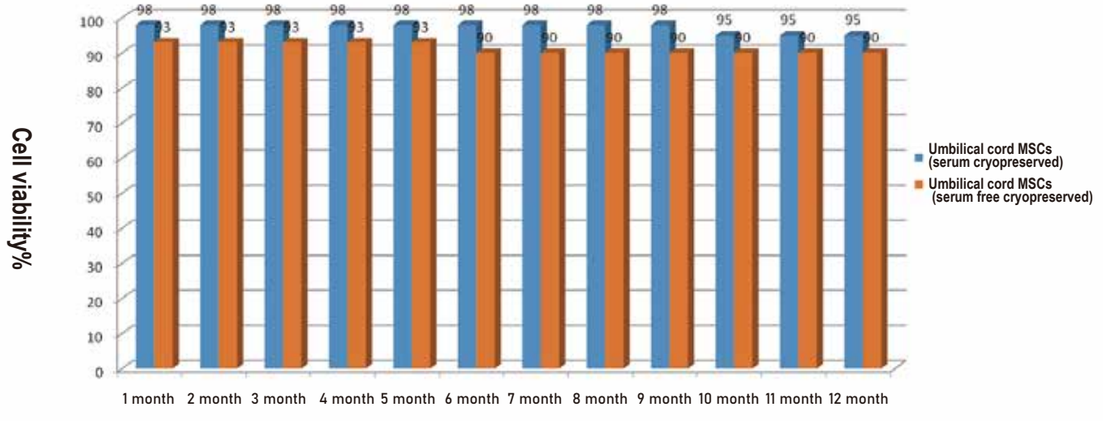
Comparison of viability of monocytes stored for different months



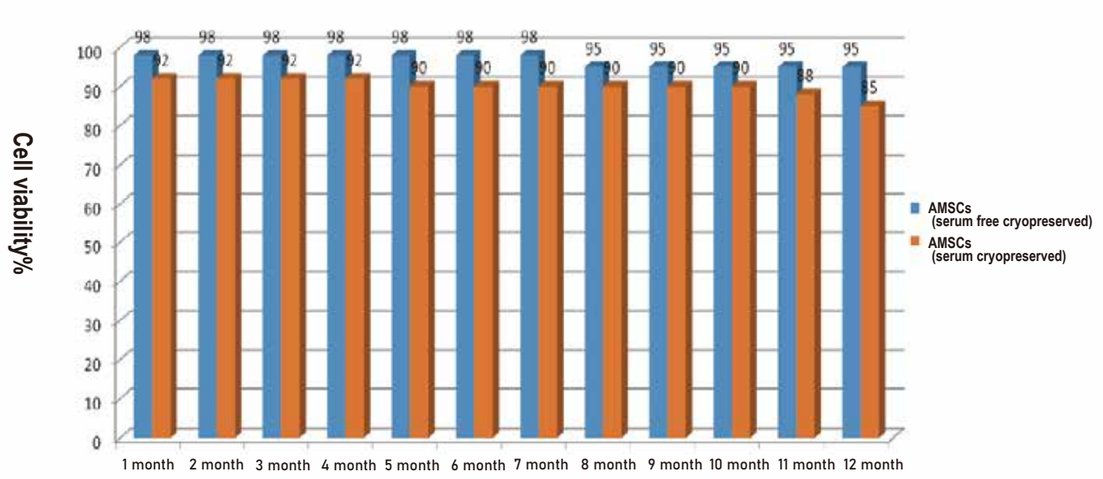
MSC cryopreservation

cells	1 month	2 month	3 month	4 month	5 month	6 month	7 month	8 month	9 month	10 month	11 month	12 month
Umbilical cord MSCs (serum free cryopreserved)	98%	98%	98%	98%	98%	98%	98%	98%	98%	95%	95%	95%
Umbilical cord MSCs (serum cryopreserved)	93%	93%	93%	93%	93%	90%	90%	90%	90%	90%	90%	90%
AMSCs (serum free cryopreserved)	98%	98%	98%	98%	98%	98%	98%	95%	95%	95%	95%	95%
AMSCs (serum cryopreserved)	92%	92%	92%	92%	90%	90%	90%	90%	90%	90%	88%	85%

Comparison of viability of umbilical cord MSCs stored for different months



Comparison of viability of AMSCs stored for different months

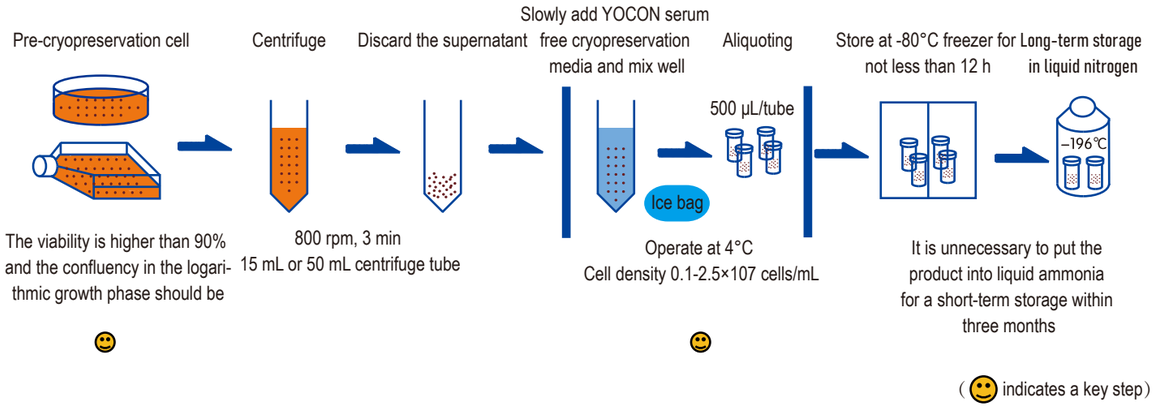


MSC cryopreservation

Study design

MSCs, the most widely used stem cells, were cryopreserved in different types of cell cryopreservation media, and then recovered, cultured, and tested f-or the phenotype of MSCs under different conditions using flow cytometry to describe the actual performance of different types of serum free cell cryopr-eservation media

Cryopreservation procedures



(indicates a key step)

Cell recovery data after cryopreservation

Type of cryopreservation media	Traditional cell cryopreser-vation media	YOCON Serum Free Cell Cryopreservation Media	Japanese Q Serum Free Cell Cryopreservation Media
MSC cell recovery rate	80%	95%	95%

Flow cytometric test results of cell phenotype

List of phenotype comparison of MSCs before cryopreservation and after cryopreservation in different cryopreservation media

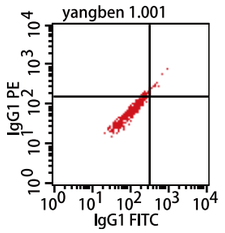
Phenotyping		CD73	CD105	CD45	CD34
Before cryo-preservation	MS phenotyping	99.87%	97.03%	0.07%	0.06%
After cryo-preservation	Traditional cryopreservation media	99.16%	95.48%	0.01%	0.12%
	Japanese cryopreservation media	99.85%	98.26%	0.10%	0.06%
	YOCON serum free cryopreservation media	99.57%	96.88%	0.06%	0.18%

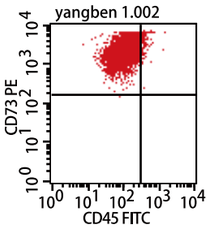
CD73>95%, CD105>95%; CD45<5%, CD34<5%; typical phenotype of MSC cells

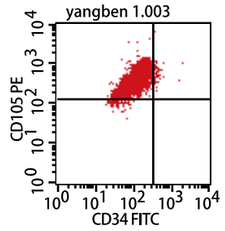
If the phenotype of the cryopreserved cells meets the above indicators, it can be determined that the cryopr-eservation and recovery processes have no effect on the phenotype of MSCs

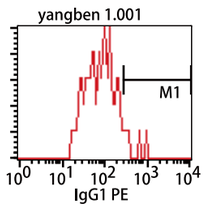
Flow cytometry

Flow cytometry test report-MSCs before cryopreservation



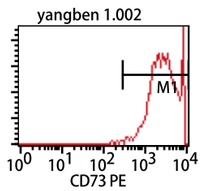






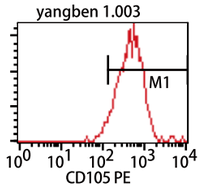
File: yangben 1.001 Gate: G1
X Parameter: IgG1 PE (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	75.86	74.99	121
M1	1.66	48.57	309.20	291



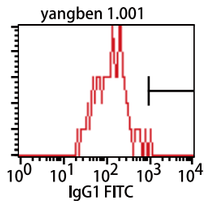
File: yangben 1.002 Gate: G1

Marker	% Gated	CV	Median	Peak Ch
All	100.00	58.65	2246.79	6792
M1	99.87	58.52	2246.79	6792



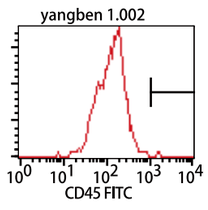
File: yangben 1.003 Gate: G1
X Parameter: CD105 PE (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	61.98	414.18	491
M1	97.03	59.99	425.51	491



File: yangben 1.001 Acquisition Date: 11-Aug-16
Gate: G1 X Parameter: IgG1 FITC (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	67.53	116.52	130
M1	0.00	***	***	***



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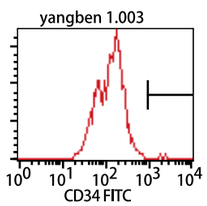
X Parameter: CD45 FITC (Log)

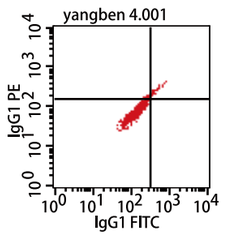
Marker	% Gated	CV	Median	Peak Ch
All	100.00	62.61	128.64	174
M1	0.07	4.70	1407.46	1309

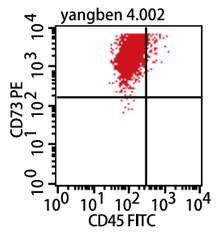


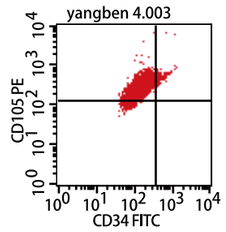
File: yangben 1.003 Gate: G1
X Parameter: CD34 FITC (Log)

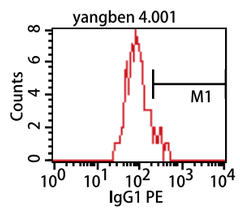
Marker	% Gated	CV	Median	Peak Ch
All	100.00	62.70	128.64	168
M1	0.06	15.83	1835.15	1639

Flow cytometry test report-MSCs before cryopreservation



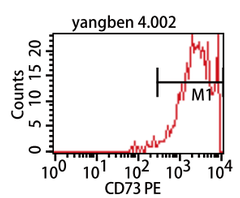






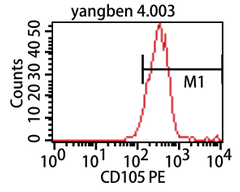
File: yangben 4.001 Gate: G1
X Parameter: IgG1 PE (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	58.54	77.74	64
M1	3.57	19.57	266.55	313



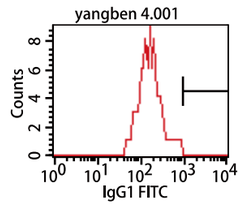
File: yangben 4.002 Gate: G1

Marker	% Gated	CV	Median	Peak Ch
All	100.00	63.09	2109.69	1715
M1	99.16	62.28	2128.75	1715



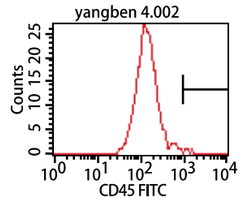
File: yangben 4.003 Gate: G1
X Parameter: CD105 PE (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	66.72	289.03	296
M1	95.48	64.73	294.27	296



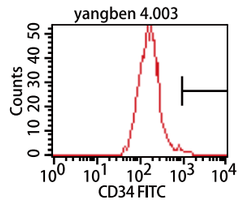
File: yangben 4.001 Acquisition Date: 11-Aug-16
Gate: G1 X Parameter: IgG1 FITC (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	54.42	149.89	179
M1	0.00	***	***	***



File: yangben 4.002 Gate: G1
X Parameter: CD45 FITC (Log)

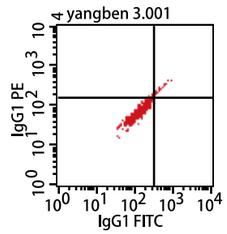
Marker	% Gated	CV	Median	Peak Ch
All	100.00	60.63	124.09	110
M1	0.11	11.67	1165.22	1036

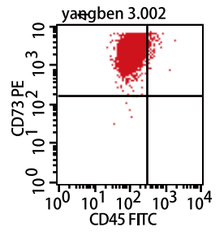


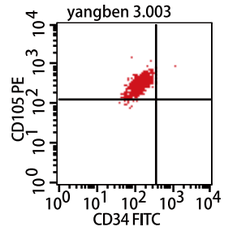
File: yangben 4.003 Gate: G1
X Parameter: CD34 FITC (Log)

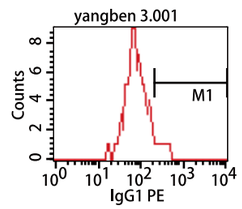
Marker	% Gated	CV	Median	Peak Ch
All	100.00	56.21	147.22	133
M1	0.12	10.28	1202.48	982

Flow cytometry test report-MSCs after cryoprese yopreservation media



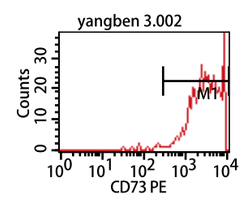






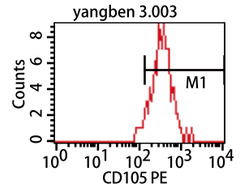
File: yangben 3.001 Gate: G1
X Parameter: IgG1 PE (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	56.84	70.41	69
M1	2.10	22.50	271.39	259



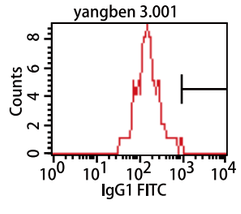
File: yangben 3.002 Gate: G1

Marker	% Gated	CV	Median	Peak Ch
All	100.00	56.13	2617.99	6792
M1	99.57	55.68	2641.65	6792



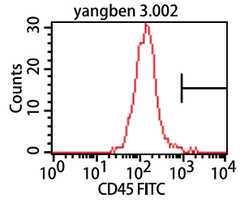
File: yangben 3.003 Gate: G1
X Parameter: CD105 PE (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	44.87	311.99	259
M1	96.88	43.00	316.23	259



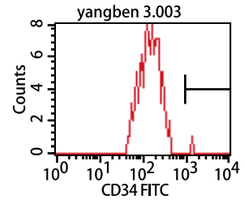
File: yangben 3.001 Acquisition Date: 11-Aug-16
Gate: G1 X Parameter: IgG1 FITC (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	55.86	134.56	133
M1	0.00	***	***	***



File: yangben 3.002 Gate: G1
X Parameter: CD45 FITC (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	58.44	125.21	125
M1	0.06	21.46	1526.14	1309



File: yangben 3.003 Gate: G1
X Parameter: CD34 FITC (Log)

Marker	% Gated	CV	Median	Peak Ch
All	100.00	53.99	127.49	110
M1	0.18	***	1197.09	1197