

SERUM FREE CELL CRYOPRESERVATION PRODUCTS

| Nomenclature | Cat No. | Specification and storage | Use and Description |
|---------------------------------|----------|---|---|
| Stem Cell Mild Digestive Enzyme | NC1004.1 | 100 mL/vial Store at 2-8°C Expiry date: 12 months | It is a gene recombinase, and is expressed in E. coli. It has no human-derived or animal-derived components. It is specially used for passage digestion of stem cell, has mild effect and can avoid over-digestion of stem cells to significantly reduce the culture failure rate of stem cells |
| Stem Cell Mild Digestive Enzyme | NC1004.2 | 500 mL/vial Store at 2-8°C Expiry date: 12 months | |

MSC SERUM FREE CULTURE RELATED PRODUCTS

| Nomenclature | Cat No. | Specification and storage | Use |
|---|----------|---|---|
| MSC Serum Free Basal Medium | NC1013 | 500 mL/vial Store at 2-8°C Expiry date: 12 months | 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. This product can only be used after adding the corresponding medium additives |
| MSC Serum Free Medium Supplement 1 (Separation of Umbilical Cord-Primary Cells and Construction of Seed Bank) | NC0103.S | 500 mL/vial Store at 2-8°C Expiry date: 12 months | This product should be used in conjunction with the MSC serum free basal medium 500 mL media can be added per 5 mL |
| MSC Serum Free Medium Supplement 2 (Passage of Umbilical Cord-Cryopreserved Cells and High-Passage Cells) | NC0105.S | | This product should be used in conjunction with the MSC serum free basal medium 500 mL media can be added per 5 mL |
| MSC Serum Free Medium Supplement 3 (Adipose-Primary Cell Separation and Subculturing) | NC0104.S | | This product should be used in conjunction with the MSC serum free basal medium 500 mL media can be added per 5 mL |
| GMP Cell Cryopreservation Media | NC1010 | 500 mL/vial Store at 2-8°C Expiry date: 12 months | It has no protein, DMSO, or human-derived or animal-derived components and is more suitable for clinical study. It supports storage of high-density cryopreserved stem cells and immune cells |

Stem Cell Mild Digestive Enzyme Specialized for Passage Digestion of Stem Cells



- 15-minute digestion does not affect its activity and subsequent passages
- It does not need to be terminated after digestion, and can be directly diluted
- 3.Higher cell viability and higher cell count

It significantly reduces the culture failure rate of stem cells

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Performance comparison

Use

It is specially used for passage digestion of stem cells, including umbilical cord MSCs, AMSCs, embryonic stem cells (ES), etc
 It has extremely mild digestion and has the minimal damage to cells. It is the latest product in the field of stem cell clinical study and clinical application
 This product is especially suitable for enterprises for mass production of stem cells. It eliminates the biggest hidden danger in culture failure of stem cell - excessive digestion
 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

Reasons for development

It is extremely difficult to accurately control the quick-cleavage digestion time during the stem cell culture due to personnel operating experience or objective operating time constraints, and excessive digestion is the main cause for culture failure of stem cells. A digestion product that is not affected by the excessive digestion should be developed to significantly reduce the culture failure rate of stem cells. Thus, mass production of stem cells will be achieved

Product advantages

Product fault tolerance is excellent
 There is little effect on subsequent passage of stem cells even the digestion time is as long as 15 minutes, which reduces requirements for operator skills
 Ready-to-use, no need of waiting
 It can be stored at 4°C for one year, and no longer needs to be stored -20°C
 It is purely artificially synthesized, and contains no animal-derived components
 There is no possibility of infection with mad cow virus (natural bovine trypsin) and foot-and-mouth disease virus (natural porcine trypsin) The ingredients are clear, and there are no unknown consequences

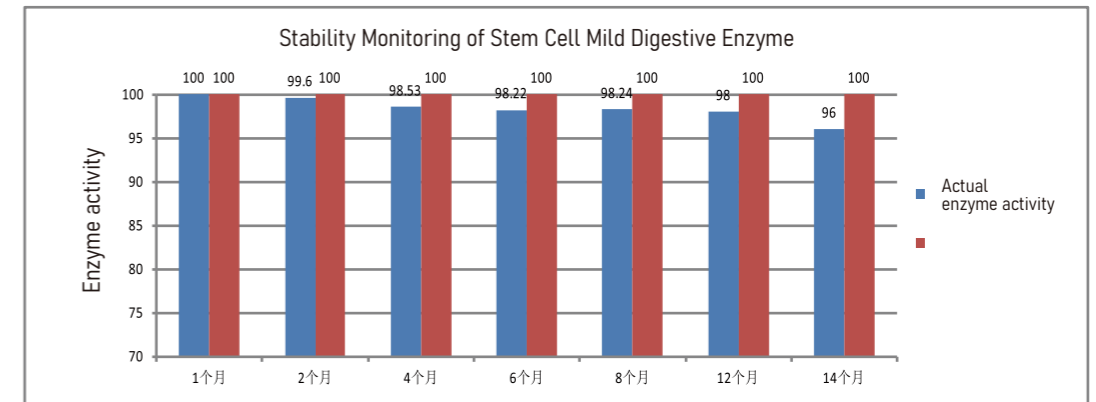
Market Share

| | |
|-------|--|
| 300 | More than 300 stem cell and immune cell customers nationwide |
| >10 | Many users of national stem cell clinical study bases |
| >10 | Selection of many companies applying for clinical stem cell drugs |
| 10000 | Validation of over 10,000 samples |
| 10 | More than 10 technicians nationwide for cooperating with customers in tests |
| 90% | Past product performance of companies with more than 90% market share in the field of virus sampling |
| 13 | A 13-year-old company |
| ∞ | We are worthy of your trust |



Features

It can be stably stored at 4°C for one year, and no longer needs to be stored at -20°C



The stem cell mild digestive enzyme has been stored at 4°C for 14 months, and the enzyme activity remained at 96% after two months beyond the expiry date

The operation is more convenient, and it is not necessary to terminate the digestion after digestion, and the product can be used only by being diluted with the culture supernatant

The traditional trypsin requires additional serum, complete medium, or trypsin inhibitors to terminate the digestion combined with the digestion site of trypsin after cells are digested
 In the design principle, the stem cell mild digestive enzyme does not need to terminate the digestion after cells are digested, **but only needs to add the culture supernatant** to dilute the digestive enzyme, and remove it by centrifugation
 Thus, the cost of use is significantly reduced. (Note: PBS can be added to dilute the stem cell mild digestive enzyme, but the cell yield will decrease by 10%-40% after centrifugation)

It is safer since the ingredients are clear without any animal-derived components. It is more suitable for clinical study, clinical application or drug application

The traditional trypsin is obtained by purification and crystallization of the bovine or pig and goat pancreas. Animal origin and extraction process determine that traditional trypsin is difficult to avoid infection with animal viruses, such as mad cow virus, swine foot-and-mouth disease virus and other unknown viruses. Therefore, it is difficult to apply to clinical related fields such as biopharmaceuticals and cell therapy
 The stem cell mild digestive enzyme is a fully artificial synthetic enzyme with definite digestion sites, is expressed in prokaryotic or eukaryotic systems. No animal-derived components are introduced in the manufacturing process, so it is extremely safe, especially suitable for clinical related fields such as biopharmaceuticals and cell therapy

Product principle

The traditional trypsin indiscriminately cleaves the arginine and lysine C-terminal of a protein to form peptide bonds
 The digestion process is classified as "non-specific digestion"
 Trypsin will continue to digest all proteins once it comes into contact with the cells until the cells become fragments
 The stem cell mild digestive enzyme specially constructed by Yocon Biology has definite action sites, and only specifically digest the cell matrixes between cells and cells and between cells and culture flasks
 It rarely digests extracellular proteins, and is classified as "specific digestion"
 Even if the stem cells are soaked in mild enzyme for 15 minutes, the cell viability can still maintain more than 90%

New-Generation Digestion Product

Comprehensive comparison of the new-generation digestion products (stem cell mild digestive enzyme) and traditional digestion product (porcine and bovine natural trypsins)

| Type | | Subtype | Traditional product | Stem Cell Mild Digestive Enzyme |
|-----------------------|--|--|-------------------------|---------------------------------|
| Digestion performance | Normal digestion (without excessive digestion) | Cell viability | 83% | above 95% |
| | | The cell count obtained by continuing to culture after digestion | 1.86×10^6 | 1.94×10^6 |
| | Digest overtime (excessive digestion) | Cell viability | 60% | above 95% |
| | | The cell count obtained by continuing to culture after digestion | 1.07×10^6 | 1.88×10^6 |
| | Extreme digestion (15-minute digestion) | Cell viability | 0-10% | above 90% |
| | | The cell count obtained by continuing to culture after digestion | Unable to harvest cells | 1.78×10^6 |

| | | | |
|-----------------------|--|---|---|
| Operation convenience | Does it need to be terminated after digestion? | Required | No |
| | Does it need to be stored at -20°C? | Required | No |
| | Is it ready-for-use? | No | Yes |
| Operation convenience | Cost of digestion termination | High. Serum, complete media or trypsin inhibitors are required | Digestion does not need to be terminated. It can be used by being diluted with the culture supernatant. |
| | Cost of virus test before cell application | High. Animal viruses such as swine foot-and-mouth disease and bovine mad cow disease need to be tested. | No test is required since there is no animal source. |

| | | | |
|---|--|---|--|
| Product safety | Is it free of animal-derived components? | No | Yes |
| | Is it possible to confirm that there is no animal virus? | No | Yes |
| Difficulty in the application of cell drugs | Confirmed difficulty affected by product residues | Extremely high Impurity components are unknown and difficult to prove | Low. The ingredients are clear, all of which are artificially synthesized components |

*The above data are the corresponding data on P4 cells obtained passaging P3 cells harvested after digesting the P2 seed cells inoculated with umbilical cord-derived MSCs in T25 culture flasks at the seeding density of 8000 cells/cm² after 72 hours

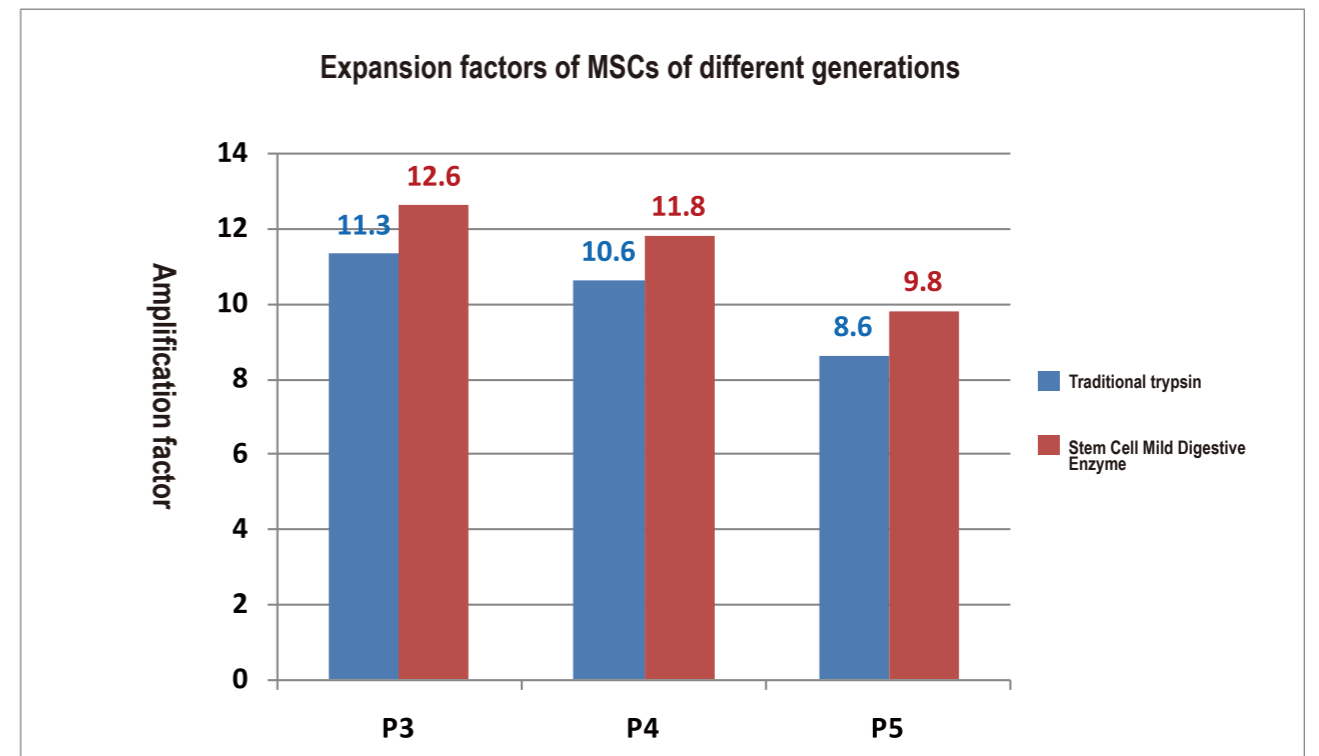
Normal digestion

P2 MSC seed cells were subcultured for 72 hours and then digested with mild enzyme and traditional trypsin, respectively, to obtain P3 MSCs, and the cell viability after digestion was detected.

P3 MSC cells were further cultured for 72 hours, digested with mild enzyme and traditional trypsin, respectively, to harvest P4 MSC cells, and the cells were counted.

| Type | Traditional trypsin | Mild digestive enzyme |
|-----------------------------|--------------------------|--------------------------|
| P3 MSC cell Viability | 85% | 95% |
| P3 MSC cell count harvested | 2.06×10^6 cells | 2.28×10^6 cells |
| P3 MSC cell Viability | 83% | 96% |
| P4 MSC cell count harvested | 2.06×10^6 cells | 1.94×10^6 cells |

P2 MSC seed cells were recovered, passaged for 3 consecutive generations, and digested with mild enzyme and traditional trypsin, respectively. Cell counts: each generation of mild enzyme was 11-14% higher than traditional trypsin.



Digest overtime

The MSC cells were digested for 2 minutes over time, respectively, and then subcultured for 72 hours, and the cells were harvested and counted: The cell count harvested by mild enzyme was 237.5% higher than that of the traditional trypsin, and the cell morphology was good under the microscope. However, the cells that were digested with traditional trypsin showed a messy morphology under the microscope after culture

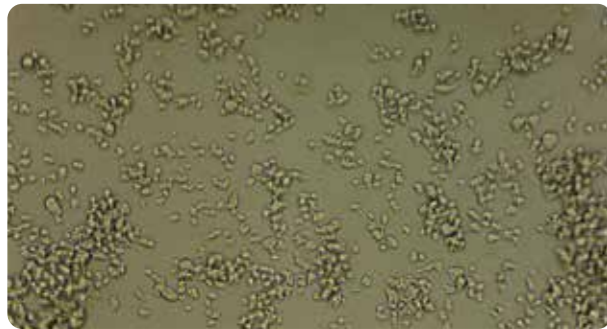
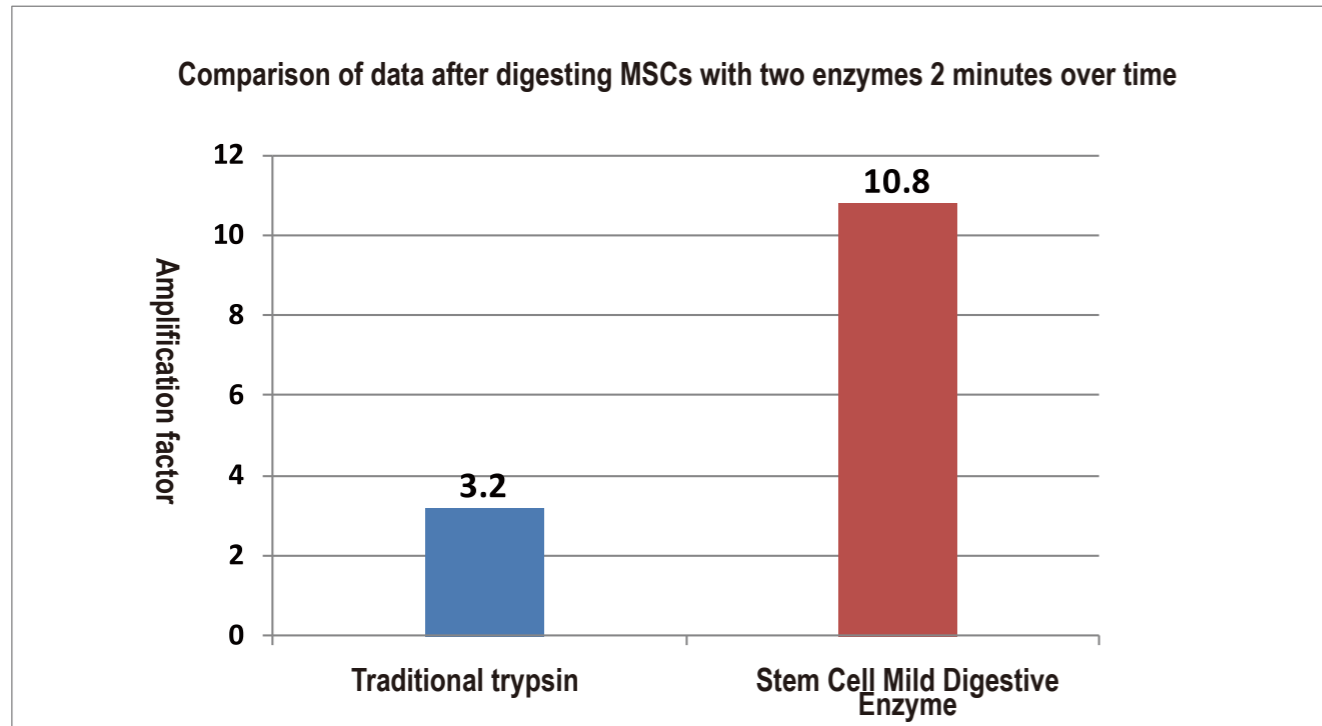


Photo of MSCs after 2-minute extended digestion by the traditional trypsin



Photo of 72 h passaging after digestion by the traditional trypsin

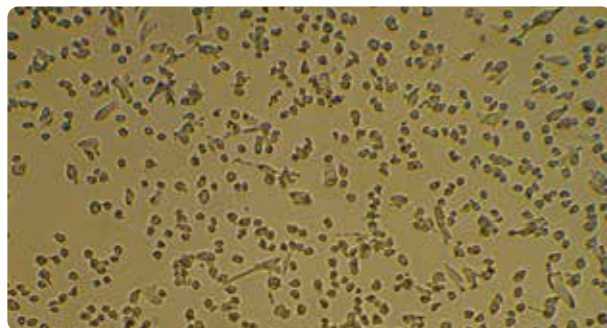


Photo of MSCs after 2-minute extended digestion by the mild enzyme

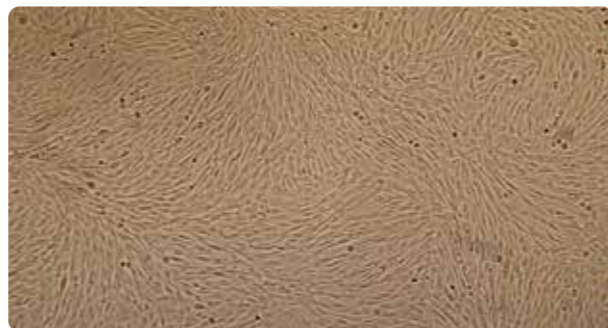


Photo of 72 h passaging after digestion by the mild enzyme

Extreme digestion

The MSC cells were digested for 15 minutes, respectively, and then subcultured for 72 hours, and the cells were harvested and counted: The cell count harvested by mild enzyme was 23 times higher than that of the traditional trypsin, and the cell morphology was good under the microscope. However, the cells that were digested with traditional trypsin showed a messy morphology under the microscope after culture

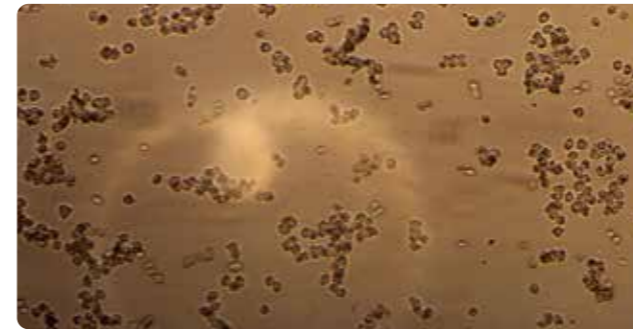
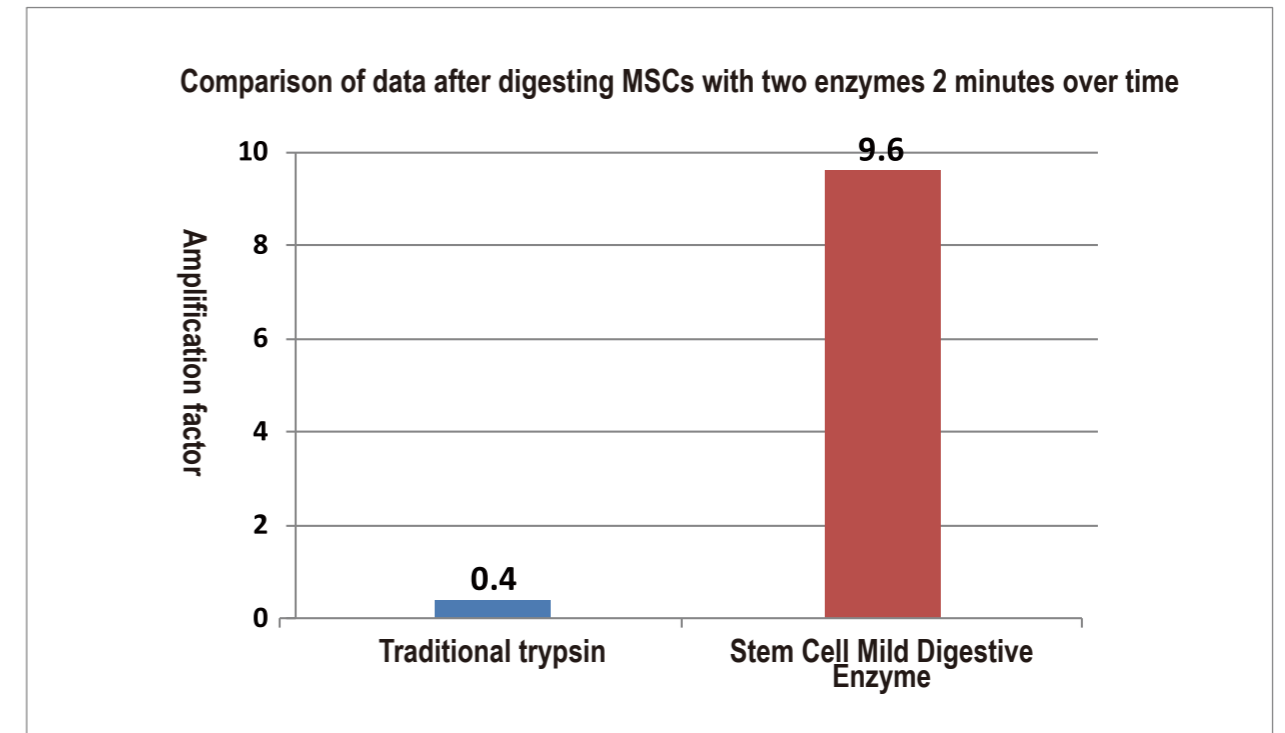


Photo of MSCs after 2-minute extended digestion by the traditional trypsin

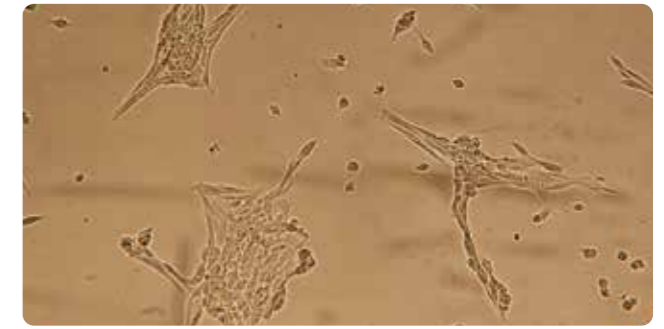


Photo of 72 h passaging after digestion by the traditional trypsin



Photo of MSCs after 2-minute extended digestion by the mild enzyme

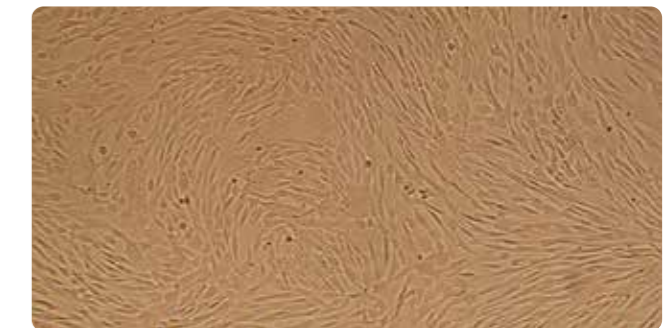


Photo of 72 h passaging after digestion by the mild enzyme