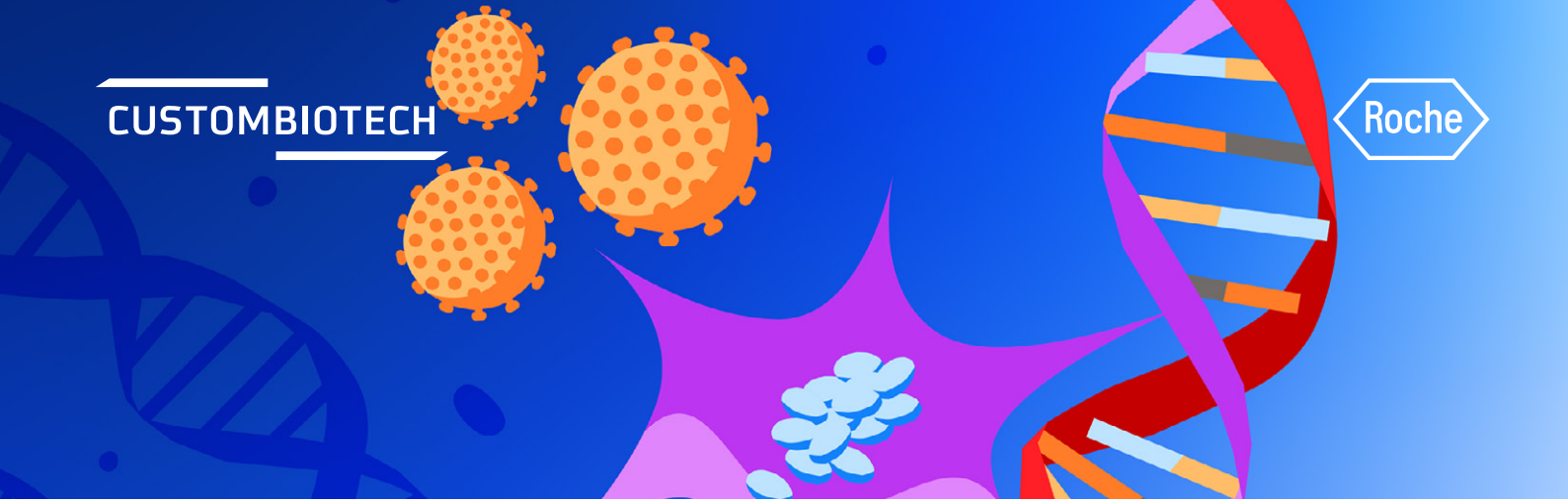

Use of Roche Recombinant Trypsin for cell culture applications

Application Note

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Introdution

Since 2004, Roche has been manufacturing recombinant trypsin (Roche Recombinant Trypsin) expressed in Pichia pastoris. The enzyme is produced in accordance to good manufacturing practice (GMP) guidelines. No animal-derived products are used in the fermentation, purification, or final formulation. As the enzyme is frequently used in the manufacturing of Active Pharmaceutical Ingredients, its production process is audited by a leading insulin manufacturer.

In addition, there is increasing demand to replace native enzymes in all manufacturing steps of products intended for use in humans. In this application note, we demonstrate the use of Roche Recombinant Trypsin in cell culture applications, such as the detachment of adherent cells, in particular with cell lines used in vaccine production.

To this end, reaction buffer components and concentration of Roche Recombinant Trypsin were first optimized to achieve quantitative detachment of MRC-5 cells without any cell damage. MRC-5 cells were chosen for these experiments because this cell line is known to be particularly sensitive to cell damage during the detachment procedure.

In a second step, we optimized the protocol for a range of other cell lines, including FRhK-4, Vero, MDCK and CHO cells, which are commonly used by pharmaceutical companies for vaccine production.

In a third step, we determined thermal stability of the diluted Roche Recombinant Trypsin solution.

Materials and Methods

Cell lines, reagents and consumables

Cell lines

Cell line	Source	Culture media
MRC-5	ATCC, Mediagnost	Hank's/Earles (1:1), 10% FCS,1% PS, 1% NaPy, 1% NEA
MDCK	Mediagnost	DMEM, 10% FCS, 1% PS, 1% NaPy, 1% NEA
FRhK-4	Prof. Dr. Bertram Flehmig, University of Tuebingen	Hank's/Earles (1:1), 10% FCS, 1% PS, 1% NaPy, 1% NEA
Vero	Prof. Dr. Bertram Flehmig, University of Tuebingen	Hank's/Earles (1:1), 10% FCS, 1% PS, 1% NaPy, 1% NEA
CHO	Roche, BMTU50104 Ch 1/27.04.09	DMEM/Ham's F12, 10% FCS, 1% PS, 1% NaPy, 1% NEA

Reagents

Reagent	Supplier	Catalog number	Lot-No.
Roche Recombinant Trypsin sample #6	Roche	03358658103	12857800
PBS w/o $CaCl_2$, $MgCl_2$ (DPBS)	Gibco	14190	368185
PBS w/o $CaCl_2$, $MgCl_2$	PAA	H15-002	H00209-1906
EDTA 0.5M pH 8 ultrapure	Gibco	15575	626351
Trypsin-EDTA 0.25%	Gibco	25200	318266A
Trypsin-EDTA 0.25%	PAA	L11-660	L66008-2494
Hank's	PAA	E15-838	E83808-2145
Earles	PAA	E15-825	E82509-1029
DMEM/Ham's F12	PAA	E15-813	E81309-1600
DMEM	Gibco	22320	483937
FCS	PAA	A15-511	A51106-0392
Pen/Strep (PS)	PAA	P11-010	P01007-1842
AA, non-essential (NEA)	PAA	M11-003	M00307-1836
Sodium pyruvate (NaPy)	PAA	S11-003	S00309-0713
Hematoxylin Solution, Mayer's	Sigma-Aldrich	51275	
Aquatex			

Roche Recombinant Trypsin (Roche Mat. No. 03 358 658/ Lot No. 12857800) was provided as a stock solution at a protein concentration of 70 mg/ml. The enzyme was stored in an acidic storage buffer (10 mM HCl/20 mM $CaCl_2$, pH 2.0) to prevent auto-catalytic digestion. Roche Recombinant Trypsin was stored in aliquots of 0.5 ml and 50 µl at –20°C and thawed at room temperature before use.

Consumables

Consumable	Supplier	Catalog number
6 well Plate	Nunc	140675
Tissue Culture Flask 12.5 cm^2 (25 ml)Plate	BD Falcon	353107
Tissue Culture Flask 25 cm^2 (50 ml)	Greiner Bio-One	690175
Tissue Culture Flask 75 cm^2 (250 ml)	Greiner Bio-One	658170
Tissue Culture Flask 175 cm^2 (500 ml)	Nunc	159910
2 ml Stripette pipette	Corning	4021
5 ml Stripette pipette	Corning	4051
10 ml Stripette pipette	Corning	4101
epT.I.P.S. 2-200 µL	Eppendorf	0030 000.870
epT.I.P.S 0,1-10 µL	Eppendorf	0030 000.811
epT.I.P.S 50-1000 µL	Eppendorf	0030 000.919
CryoTubes 1 ml	Nunc	3-75353
PP Tubes, 50 ml, conical	Sarstedt	62.547.254
High Clarity PP Centrifuge Tube, Conical Bottom, 15 ml	BD Falcon	352096

Protocol: Trypsinization of cell lines with Roche Recombinant Trypsin

Each cell line was grown to reach a confluent monolayer. After removal of culture media, cells were washed twice with 5.0 ml PBS, pre-warmed to 37°C. Then, 1.0 ml Roche Recombinant Trypsin diluted to $1:10^{-4}$ in PBS/0.5 mM EDTA and pre-warmed to 37°C was added. Cells were inspected under a microscope, and incubated until they were completely detached and resuspended. To stop the trypsinization process, cell suspensions were diluted with the appropriate cell culture medium (see 1.2) supplemented with 10% FCS, and centrifuged at 1,300 rpm for 10 min. Supernatants were removed and the cells resuspended in fresh culture media according to the appropriate splitting ratio as described in the Results section.

Protocol: Cell fixation and staining

Cells were seeded in 6-well plates (12.5 cm^2). After reaching confluency, cells were washed with 1 ml PBS. Control cells, which were not treated with Roche Recombinant Trypsin, were fixed with 2 ml/well 2.5% formaldehyde in PBS directly after the washing step. Cells treated with Roche Recombinant Trypsin were purified after the described incubation time (see results section) prior to fixation with 2 ml/well 2.5% formaldehyde in PBS. The fixated cells were stained by incubation with Mayer's Hematoxylin Solution for 10 min followed by a wash step with water. The fixed and stained cells were covered with Aquatex solution and photographed under the Axioskop microscope (Zeiss) at 100x magnification.

Results

Optimization of Roche Recombinant Trypsin concentration for MRC-5 cell detachment

Since 2004, Roche has been manufacturing Recombinant Trypsin in accordance to good manufacturing practice (GMP) guidelines. No animal-derived products are used in the fermentation, purification, or final formulation.

Roche Recombinant Trypsin is used in highly regulated manufacturing processes, e.g., in the production of Active Pharmaceutical Ingredients (API). The enzyme is of high purity and contains almost no detectable activity of other proteases (see Fig. 01).

Because Roche Recombinant Trypsin is provided at very high purity (see Fig. 01), we expected that an optimal efficiency of the enzyme could be obtained at a lower protein concentration compared to standard quality trypsin products. Therefore, our first approach to optimize the trypsinization protocol was to examine several protein concentrations, dilution factors, and detachment reaction buffers.

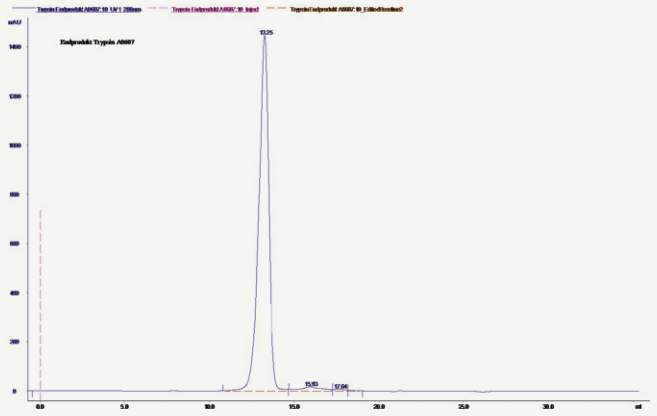
Roche Recombinant Trypsin is stored in an acidic buffer containing 10 mM HCl and 20 mM $CaCl_2$ at pH 2.0. The stock solution, provided at a concentration of 70 mg/ml, was diluted stepwise in PBS/1 mM EDTA buffer (see Table 01).

PBS buffer without $MgCl_2$ or $CaCl_2$ was used for the dilution steps because Ca^{2+} and Mg^{2+} ions in solution can promote cell aggregation. The EDTA concentration in the dilution buffer was subsequently optimized as described in section 4.

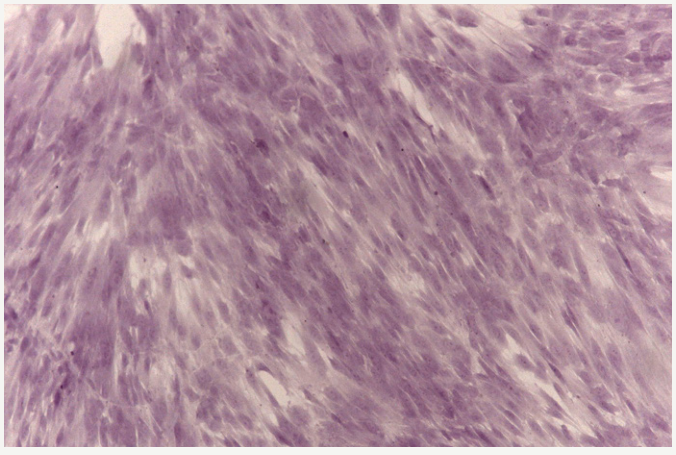
Diluted enzyme solutions were pre-heated to 37°C for about 10 min before use.

MRC-5 cells were used because this cell line is known to be particularly sensitive to cell damage during trypsinization.

Five tissue culture flasks of 25 cm^2 (50 ml) were seeded with MRC-5 cells and grown to a confluent monolayer (see Fig. 02). After removal of the culture media supernatant, cells were removed and washed twice with 5.0 ml PBS each.



01
HPLC Superdex 75™ analysis of Roche Recombinant Trypsin in final formulation buffer (Roche internal data).



02
C onfluent monolayer of MRC-5 cells. Cells were fixated and stained as described in Materials and Methods.

Thermal cycling protocol

Dilution factor	Volume Roche Recombinant Trypsin	Volume buffer (PBS + EDTA 1 mM)	Total volume	Protein concentration	Final pH
none	5 ml	0 ml	5 ml	70 mg/ml	2
1:10 (10^{-1})	0.5 ml	4.5 ml	5 ml	7 mg/ml	6.8
1:100 (10^{-2})	0.5 ml 1:10	4.5 ml	5 ml	700 µg/ml	7.4
1:1,000 (10^{-3})	0.5 ml 1:100	4.5 ml	5 ml	70 µg/ml	7.4
1:10,000 (10^{-4})	0.5 ml 1:1,000	4.5 ml	5 ml	7 µg/ml	7.4
1:100,000 (10^{-5})	0.5 ml 1:10,000	4.5 ml	5 ml	0.7 µg/ml	7.4
1:1,000,000 (10^{-6})	0.5 ml 1:100,000	4.5 ml	5 ml	0.07 µg/ml	7.4

T01
Stepwise dilution of Roche Recombinant Trypsin stock solution.

1.0 ml each of Roche Recombinant Trypsin solutions at dilutions of 10^{-6}, 10^{-5}, and 10^{-4} were added to the cells. As reference, Trypsin-EDTA products from other suppliers (PAA, Gibco) were added at a concentration of 0.25%. Because Roche Recombinant Trypsin is provided at very high purity, dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were not used in the first round of experiments. Cell detachment was observed under the microscope. Rounding of the cells was observed after 30 sec at room temperature at all three dilutions, followed by cell clumping with the 10^{-6} and 10^{-5} dilutions. No cell clumps were observed with the 10^{-4} dilution. A comparable result was observed with the two reference trypsin products at the 0.25% concentration. After incubation for 2 min at room temperature, both the 10^{-4} dilution of Roche Recombinant Trypsin and the two reference trypsin solutions yielded a homogeneous cell suspension. These five cell suspensions were added to 10 ml HE-media/10% FCS each and centrifuged for 10 min at 1,300 rpm to stop the trypsinization process and to minimize cell damage. The supernatants were removed and ¼ of the MRC-5 cells from each dilution of Roche Recombinant Trypsin and of the reference trypsin products were inoculated in fresh T25 flasks with the appropriate cell culture media. Cells were observed under the microscope after 4 h and after 1, 2, 3, and 4 days. After 4 hours, cells from all five experiments were observed to grow well, showing a healthy elongated morphology. No cell damage was observed for any setup containing any of the dilutions of Roche Recombinant Trypsin nor for the two reference trypsin products. However, at 10^{-6} und 10^{-5} dilutions of Roche Recombinant Trypsin, we observed an increase of cell clumping similar to our observation during the trypsinization process. No cell clumps formed with the 10^{-4} dilution of Roche Recombinant Trypsin. After 4 days, a confluent monolayer formed in all five trypsinization experiments.

Thermal cycling protocol

Trypsin source	Incubation time [min]	Cell suspension	Microscopy	Confluency reached after
Roche Recombinant Trypsin 10^{-6} dilution (in PBS EDTA 1 mM)	2 min RT	cell clumps	single cells and cell clumps	4 days
Roche Recombinant Trypsin 10^{-5} dilution (in PBS EDTA 1 mM)	2 min RT	cell clumps	single cells and cell clumps	4 days
Roche Recombinant Trypsin 10^{-4} dilution (in PBS EDTA 1 mM)	2 min RT	homogeneous single cells	homogeneous single cells	4 days
Trypsin competitor 1 (Trypsin EDTA 0.25%)	2 min RT	homogeneous single cells	homogeneous single cells	4 days
Trypsin competitor 2 (Trypsin EDTA 0.25%)	2 min RT	homogeneous single cells	homogeneous single cells	4 days

T02
Optimization of trypsin concentration for trypsinization of MRC-5 cells (data on file).

Our results showed that a trypsin/EDTA solution of comparatively low 10^{-4} dilution, (referred to as Roche Recombinant Trypsin 10^{-4} in subsequent text), yielded results for the detachment process of adherent cells comparable to those with 0.25% solutions of the reference Trypsin/EDTA products. This 0.25% concentration corresponds to a dilution factor of 1:28 (see results chapter “Evaluation of Roche Recombinant Trypsin at 2,5 mg/ml concentration”, p. 11).

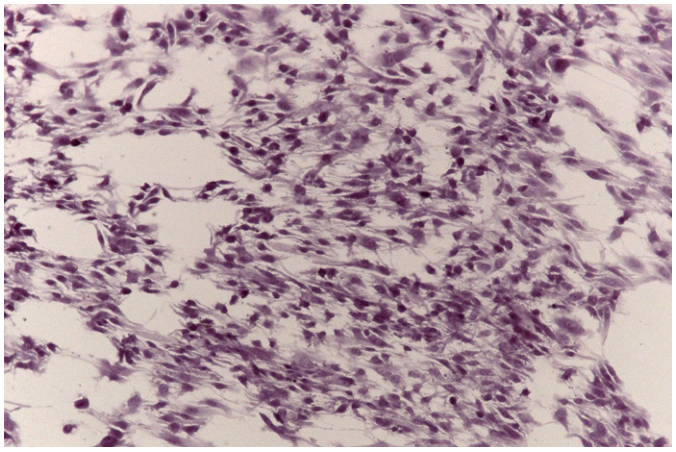
Optimization of the EDTA concentration in the PBS/EDTA dilution buffer

To optimize the EDTA concentration in the PBS/EDTA dilution buffer, a PBS/EDTA 0.5 mM stock solution was used for stepwise dilution of Roche Recombinant Trypsin in a second round of experiments (see Table 03).

Diluted Roche Recombinant Trypsin was stored at –20°C.

Dilution factor	Volume Roche Recombinant Trypsin	Volume PBS/ 0.5 mM EDTA	Total volume
1:10	50 µl	450 µl	0.5 ml
1:100	50 µl 1:10	450 µl	0.5 ml
1:1,000	50 µl 1:100	450 µl	0.5 ml
1:10,000	0.5 ml 1:1,000	4.5 ml	5.0 ml

T03
Dilution of Roche Recombinant Trypsin stock solution with PBS/0.5 mM EDTA (data on file).



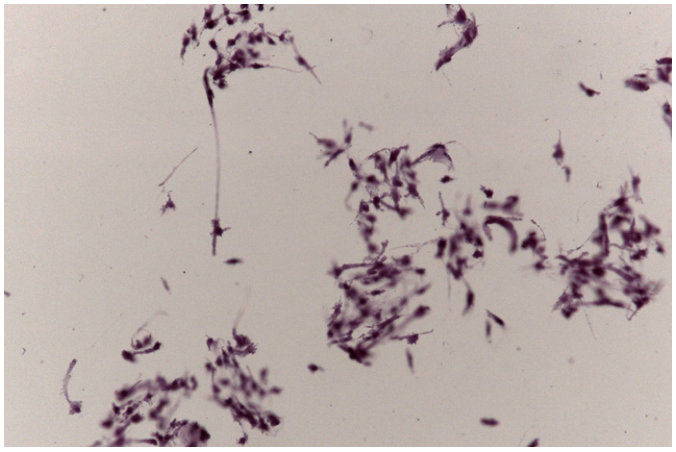
03
MRC-5 cells after 1 min incubation at room temperature with Roche Recombinant Trypsin 10^{-4} in PBS/EDTA 0.5 mM.

Tissue culture flasks of 25 cm^2 (50 ml) were seeded with MRC-5 cells and grown to a confluent monolayer (see Fig. 02). After removal of the culture media supernatant, cells were removed and washed twice with 5.0 ml PBS each.

One of the following solutions was added to each T25 flask:

- 1.0 ml Roche Recombinant Trypsin 10^{-4} in PBS/EDTA 0,5 mM
- 1.0 ml Roche Recombinant Trypsin 10^{-4} in PBS/EDTA 1 mM
- 1.0 ml Reference trypsin (PAA)

Cells were observed under the microscope as described above (see Fig. 03 and 04).



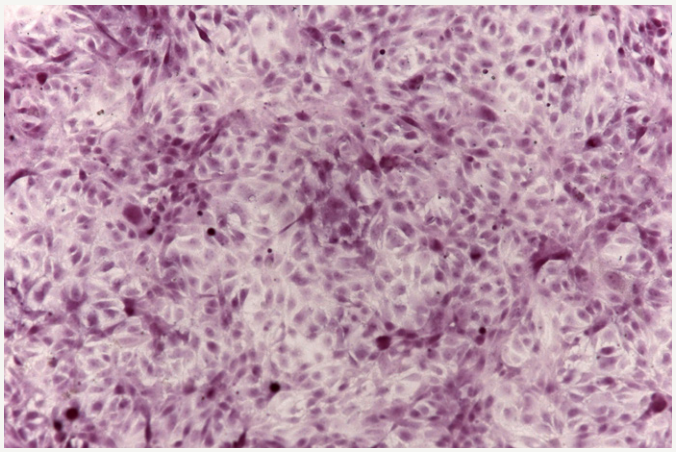
04
MRC-5 cells after 3 min incubation at room temperature with Roche Recombinant Trypsin 10^{-4} in PBS/EDTA 0.5 mM.

Trypsin source	Incubation conditions	Cell suspension	Microscopy	Confluency reached after
Roche Recombinant Trypsin 10^{-4} (in PBS EDTA 0,5 mM)	2 min RT	homogeneous	well-grown cells	4 days
Roche Recombinant Trypsin 10^{-4} (in PBS EDTA 1 mM)	2 min RT	homogeneous	well-grown cells	4 days
Reference Trypsin PAA	2 min RT	homogeneous	well-grown cells	4 days

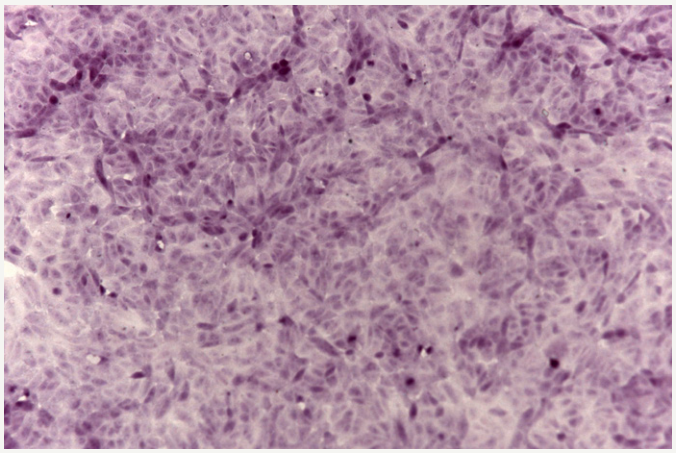
T04
Comparison of 0.5 mM and 1 mM EDTA concentrations in the dilution buffer (data on file).

No difference in the trypsinization process or in cell growth was observed when dilution buffers containing 0.5 mM EDTA or 1 mM EDTA were used (see Table 04). Therefore, the following experiments were performed with an EDTA concentration of 0.5 mM.

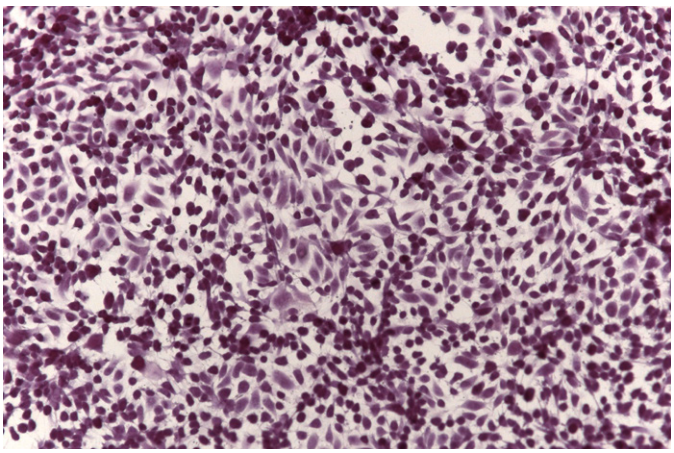
Trypsinization of FRhK-4 and Vero Cells using Roche Recombinant Trypsin 10^{-4} in PBS/EDTA 0.5 mM
Trypsinization of a confluent monolayer of FRhK-4 and Vero cells in T25 flasks (see Fig. 05 and 06) was performed as described in Materials and Methods, section 2).



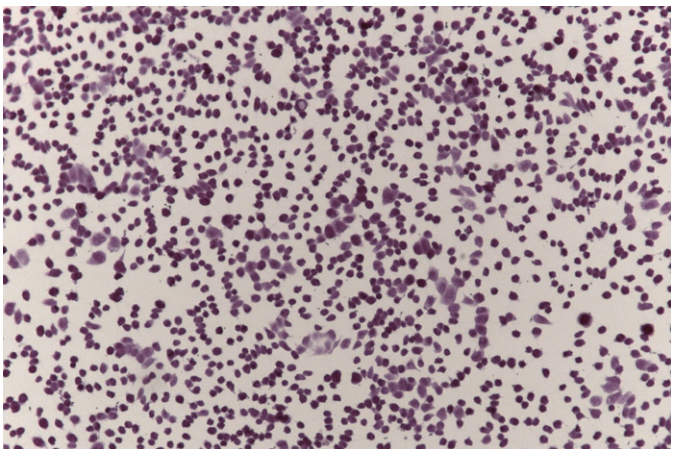
05
Confluent monolayer of FRhK-4 cells.



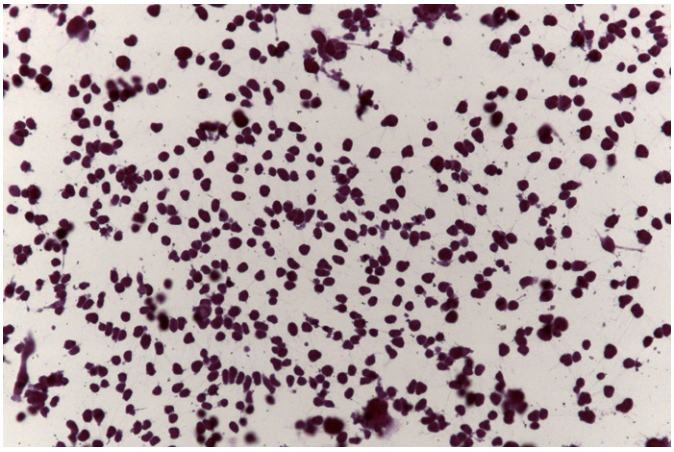
06
Confluent monolayer of Vero cells.



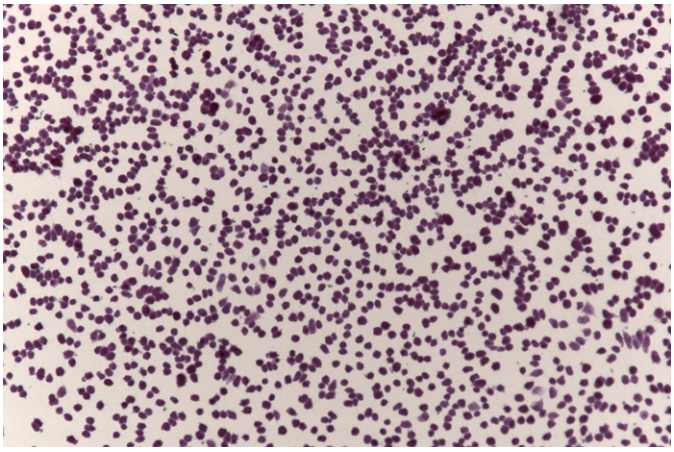
07
FRhK-4 cells after 3 min incubation at 37°C with Roche Recombinant Trypsin 10^{-4}.



08
Vero-Cells after 3 min incubation at 37°C with Roche Recombinant Trypsin 10^{-4}.



09
FRhK-4 cells after 7 min incubation at 37°C with Roche Recombinant Trypsin 10^{-4}.

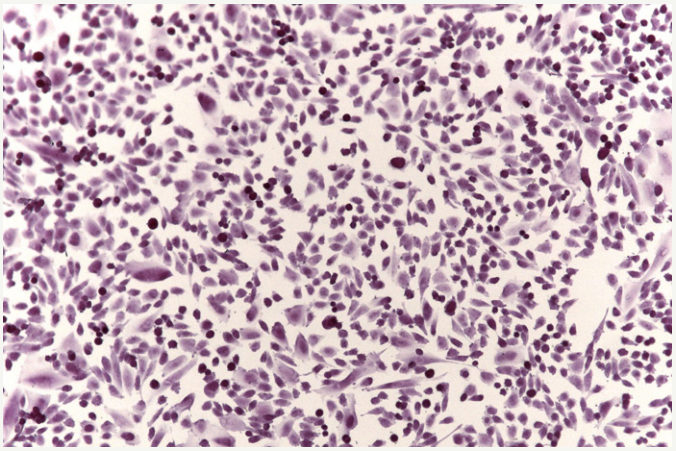


10
Vero-Cells after 7 min incubation at 37°C with Roche Recombinant Trypsin 10^{-4}.

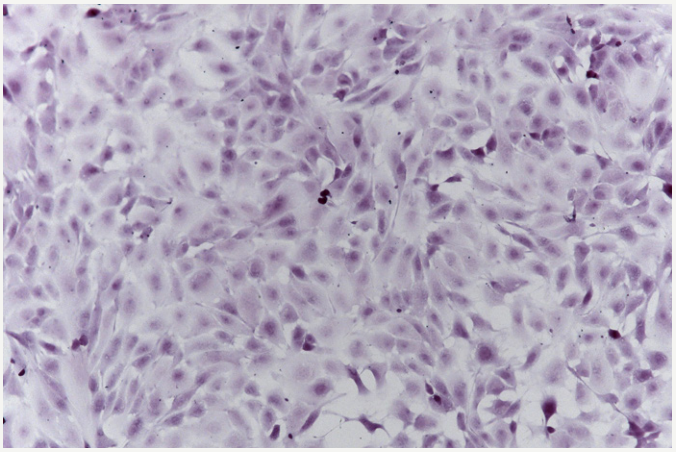
After adding 1.0 ml Roche Recombinant Trypsin 10^{-4}, cells were observed under the microscope. In contrast to MRC-5 cells, FRhK-4 and Vero cells showed cell rounding only after 3 min incubation at 37°C (see Fig. 07 and 08). After 7 min incubation at 37°C, cells were quantitatively resuspended (see Fig. 09 and 10). FRhK-4 and Vero cell suspensions were treated according to the protocol (see Materials and Methods, section 2), and 1/8 of each cell suspension was transferred to a fresh T25 flask. After 4 hours, cells from all five experiments were observed to grow well, showing a healthy elongated morphology. After 3 days, all five setups reached confluency. No differences between the cells incubated with Roche Recombinant Trypsin and the reference trypsin product were observed (data not shown).

Trypsinization of CHO and MDCK cells using Roche Recombinant Trypsin 10^{-4} in PBS/EDTA 0.5 mM (CHO cell line = BMTU 50104 Ch1/27.04.09)

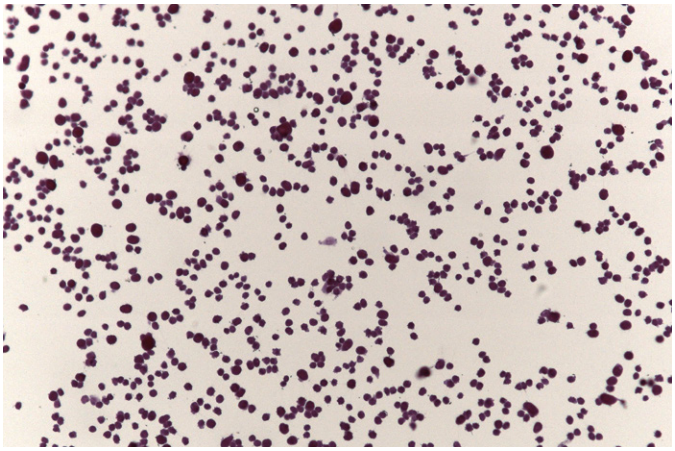
Trypsinization of a confluent monolayer of CHO and MDCK cells in T25 flasks was performed as described in Materials and Methods.



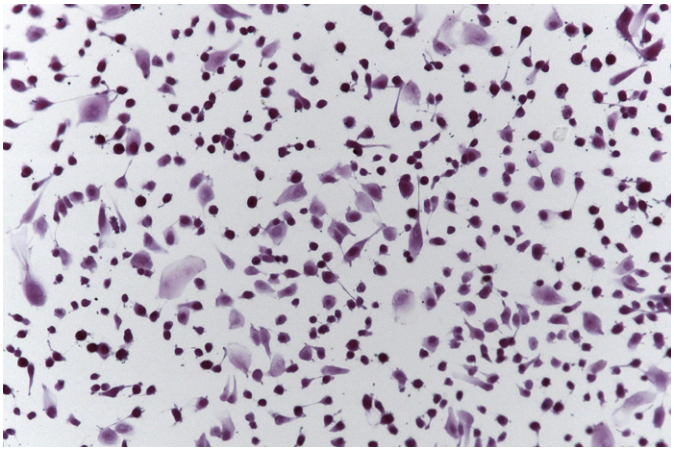
11
Confluent monolayer of CHO cells.



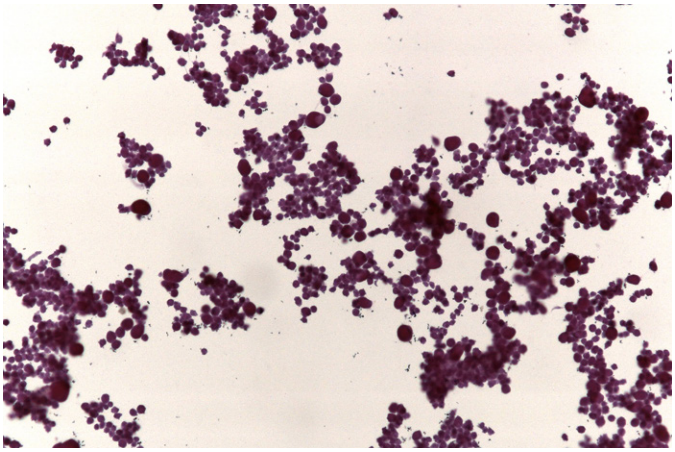
12
Confluent monolayer of MDCK cells.



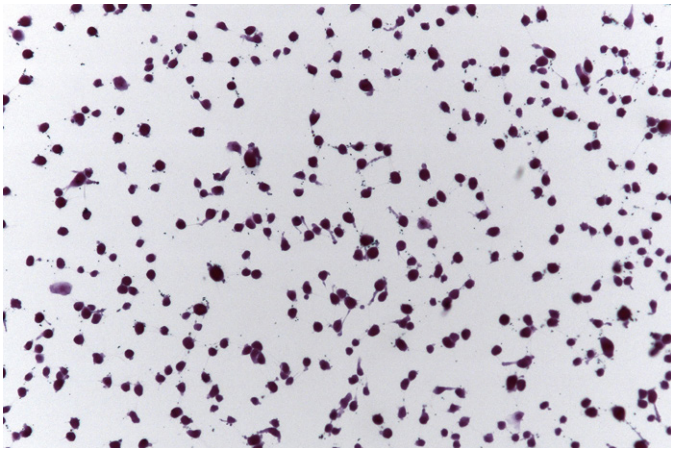
13
CHO cells after 1 min incubation at room temperature with Roche Recombinant Trypsin 10^{-4}.



14
MDCK cells after 2.5 min incubation at room temperature with Roche Recombinant Trypsin 10^{-4}.



15
CHO cells after 3 min incubation at room temperature with Roche Recombinant Trypsin 10^{-4}.



16
MDCK cells after 5 min incubation at room temperature with Roche Recombinant Trypsin 10^{-4}.

After addition of 1.0 ml Roche Recombinant Trypsin 10^{-4}, cells were observed under the microscope. CHO and MDCK cells showed cell rounding after 1 min and 2.5 min incubation at room temperature, respectively (see Fig. 13 and 14). Cells were quantitatively resuspended after 3 and 5 min incubation at room temperature, respectively (see Fig. 15 and 16).

CHO and MDCK cell suspensions were treated according to the protocol (see Materials and Methods, section 2), and 1/10 of each cell suspension was transferred to a fresh T25 flask. After 4 hours, cells from all five experiments were observed to grow well, showing a healthy elongated morphology. After 3 days, all five setups reached confluency. No differences between the cells incubated with Roche Recombinant Trypsin and the reference trypsin product were observed (data not shown).

Results of the detachment protocols for different cell lines using Roche Recombinant Trypsin are summarized in Table 05.

Cell line	Splitting ratio	Roche Recombinant Trypsin 10^{-4}	
		Incubation time in the presence of Trypsin*	Confluency reached after
MRC-5	1:4	2 min RT	4 days
Vero	1:6	7 min 37°C	3 days
FRhK-4	1:8	7 min 37°C	3 days
CHO	1:10	3 min RT	4 days
MDCK	1:5	5 min RT	3 days

T05
Detachment of various cell lines using Roche Recombinant Trypsin 10^{-4} (data on file).

Passaging of cell cultures
Using Roche Recombinant Trypsin 10^{-4}, MRC-5 cells were detached and repassaged 9 times, Vero and FRhK-4 cells were detached and repassaged 6 times, CHO cells 4 times, and MDCK cells 3 times. All cells showed consistent growth and a typical healthy morphology. Our experiments showed that the results obtained with Roche Recombinant Trypsin at a low protein concentration of 7 µg/ml (Roche Recombinant Trypsin 10^{-4}) were identical to those obtained with the more concentrated reference trypsin/EDTA solutions, and exhibited no cell damage.

* Incubation time: time needed for complete cell detachment

Evaluation of Roche Recombinant Trypsin at 2.5 mg/ml concentration
To simulate the 0.25% Trypsin/EDTA concentration commonly offered by other vendors, the 70 mg/ml stock solution of Roche Recombinant Trypsin was diluted 1:28 in PBS/EDTA 0.5 mM to obtain a concentration of 2.5 mg/ml (0.25%). This dilution was evaluated in cell detachment experiments with various cell lines and compared to the performance of Roche Recombinant Trypsin 10^{-4} (see Table 06).

Cell line	Splitting ratio	Roche Recombinant Trypsin/EDTA 0.25%		Roche Recombinant Trypsin 10^{-4}	
		Incubation time in the presence of Trypsin*	Confluency reached after	Incubation time in the presence of Trypsin*	Confluency reached after
MRC-5	1:4	0.5 min RT	5	3 min RT	4
Vero	1:6	7 min 37°C	3	7 min 37°C	3
FRhK-4	1:8	7 min 37°C	3	7 min 37°C	3
CHO	1:10	0.5 min RT	4	3 min RT	4
MDCK	1:5	2.0 min RT	3	5 min RT	3

* Incubation time: time needed for complete cell detachment

T06
Comparison of Roche Recombinant Trypsin 1:28 und 10^{-4} (data on file).

By using the 0.25% Roche Recombinant Trypsin/EDTA solution, the incubation time needed to reach complete cell detachment could be slightly shortened compared to the Roche Recombinant Trypsin 10^{-4} solution. However, the time needed until the cells reached confluency again was mostly unchanged. Since MRC-5 cells required more time to reach confluency again, we assume that the more concentrated 0,25% Roche Recombinant Trypsin solution caused more damage to cells compared to the Roche Recombinant Trypsin 10^{-4} solution. Therefore, to avoid any risk of cell damage, we recommend using the much less concentrated Roche Recombinant Trypsin 10^{-4}/EDTA solution.

Verification study Roche

For verification two different lots of Roche recombinant Trypsin was used with the following composition:

- PBS/0.5 mM EDTA buffer
- 7 microgram/ml
- pH: 7.3 ± 0.5

Read out parameters like “time to detachment”, “viability”, “doubling time” and cell morphology were compared to those received with other commonly used Trypsin products.

Experimental set up:

- Cells were cultivated in 6-well plates or 25 cm^2 cell culture flasks using their respective media and culture conditions
- Confluent cultures were washed with PBS followed by incubation with Roche Rec. Trypsin and Accutase® fromvPAA: ~ 1 ml/25 cm^2
- After detachment Trypsin was neutralized with respective cell culture medium: ~ 3 Vol.
- Cells were centrifuged at 200 x g for 3 min, supernatant was discarded and cells were re-suspended in respective media.
- After appropriate splitting, cells were further cultivated until they reached 80% confluence

	Roche Trypsin rec. (ready to use; final reagent composition containing PBS/0.5 mM EDTA buffer with 7 microgram/ml Roche Trypsin							
	Time to detachment [min]		Viability [%]		Expansion [No. of passages]		Doubling time [days]	
Cell line	Trypsin rec.	Compe-titor	Trypsin rec.	Compe-titor	Trypsin rec.	Compe-titor	Trypsin rec.	Compe-titor
hMSCs	3	3	95	91 (Acc.), 92 (Tryp.LE)	p3	p3	2.9	3.3 (Acc.), 2.9 (TrypLE)
iPS	3	3	95	89	p3	p3	–	
MDA	3	3	88	82	p4	p4	3.3	2.7
BT474	3	3	92	84	p3	p3	7.9	7.5
HCT116	3	3	96	96	p4	p4	0.9	0.9

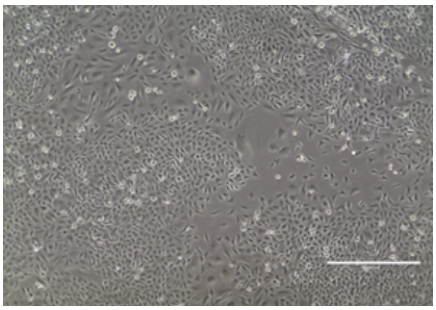
T07
Performance Data of Roche Recombinant Trypsin (Pilot lot 1, ready to use) (data on file).

	Roche Trypsin rec. (ready to use final reagent composition containing PBS/0.5 mM EDTA buffer with 7 microgram/ml Roche Trypsin)							
	Time to detachment [min]		Viability [%]		Expansion [No. of passages]		Doubling time [days]	
Cell line	Trypsin rec.	Compe-titor	Trypsin rec.	Compe-titor	Trypsin rec.	Compe-titor	Trypsin rec.	Compe-titor
HEK293	2	2	96	95	p5	p5	1,3	1,4
HT1080	2	2	97	97	p5	p5	0,8	0,8
NIH3T3	3	3	96	94	p5	p5	1,4	1,3
HCT116	2	2	97	97	p5	p5	0,7	0,7
BT474	3	3	94	93	p5	p5	4	4
CHO	1,5	1,5	98	98	p5	p5	0,6	0,6
ipS	3	3	94	93	p5	p5	1,1	1,0

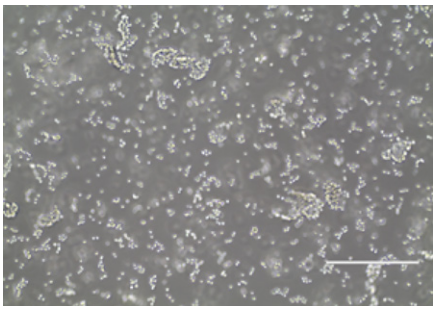
T08
Performance Data of Roche Recombinant Trypsin (Pilot lot 2, ready to use) (data on file).

Cell Morphology
Normal Morphology of cells using Trypsin rec. cell culture grade as passaging reagent.

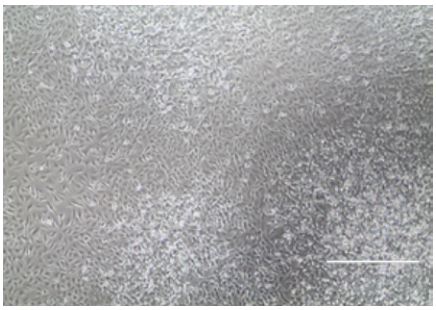
CHO cells



CHO cells prior to split

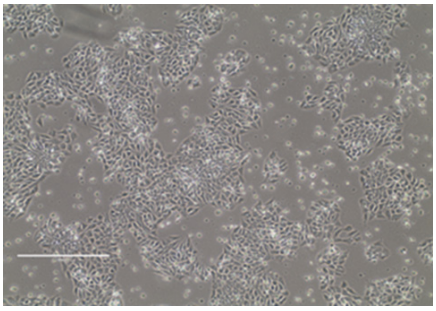


1,5 min. Trypsin rec. cell culture grade incubation

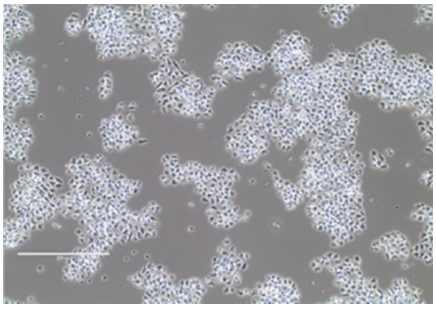


Morphology of CHO cells at p4

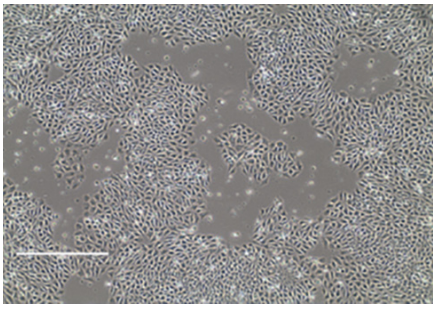
Inducted pluripotent stem cells (iPs)



iPs cells prior to split

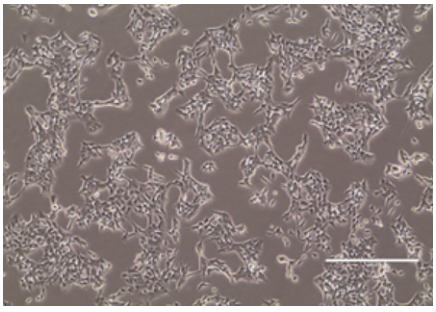


3 min. Trypsin rec. cell culture grade incubation

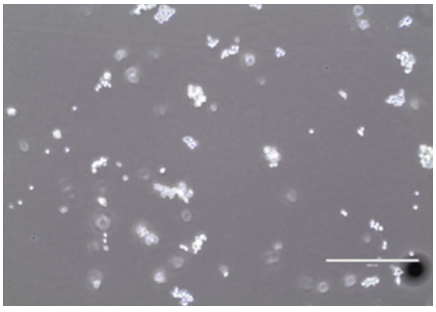


Morphology of iPs at p4

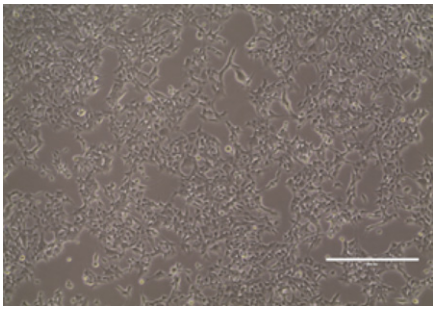
HEK 293



HEK 293 cells prior to split



2 min. Trypsin rec. cell culture grade incubation



Morphology of HEK 293 at p4

Thermal stability of Roche Recombinant Trypsin 10^{-4}

The stability of the diluted enzyme (7 µg/ml in PBS/0.5 mM EDTA buffer at pH: 7.3 ± 0.5) has been proven at Roche according to DIN/ISO 13485 (data not shown). Storage and shipping is recommended at -20°C. The diluted enzyme is considered to be stable:

- for 24 months at -20°C (-15 to -25°C)
- after thawing, at 4° (2 to 8°C) for 24 months
- at room temperature (25°C) for 24 hours

Conclusion

Roche Recombinant Trypsin was successfully used for the detachment of adherent cell lines. To show the broad application range of this enzyme, we evaluated various cell lines (e.g. MRC-5, FRhK-4, Vero, CHO, and MDCK) which are frequently used for manufacturing of pharmaceutical products. To dilute the enzyme stock solution, we used a PBS/EDTA 0.5 mM buffer. We could demonstrate that the high-purity Roche Recombinant Trypsin has high enzymatic activity even at a very low concentration of 7 µg/ml (10^{-4} dilution of the stock solution of 70 mg/ml) for all cell lines tested (see Table 05). Using this diluted solution, we obtained results identical to those achieved with commercially available trypsin/EDTA solutions, which are offered at a significantly higher concentration of0.25% (Gibco, PAA/see Table 02). While incubation times and temperatures vary between the different cell lines, the same conditions for each cell line can be used both with the Roche Recombinant Trypsin and the reference trypsin/ EDTA solutions.

Experiments using a 0.25% solution of Roche Recombinant Trypsin (2.5 mg/ml) showed that while incubation times needed to reach quantitative detachment and resuspension could be decreased compared to the 10^{-4} dilution, incubation times needed to reach confluency again were longer for some critical cell lines such as MRC-5 cells (Table 06).

This is possibly caused by increased cell damage at higher enzyme concentrations. On the other hand, further dilutions of the stock solution of 70 mg/ml (e.g., 10^{-5} or 10^{-6}) are not recommended, because we observed cell clumping during trypsinization and cell growth in the first round of cell passaging. With a 10^{-4} dilution of the stock solution, repeated trypsinization and passaging was possible without any detectable cell damage.

Based on our results, we recommend using Roche Recombinant Trypsin at a very low protein concentration of 7 µg/ml. With this diluted enzyme solution, we obtained results that were identical to the reference trypsin/EDTA solutions. At the same time, the risk of cell damage was significantly reduced.

Stability tests showed that Roche Recombinant Trypsin 10^{-4} is stable at this dilution when stored at 4°C for a period of 24 months without any loss of activity or performance, as analyzed in cell detachment experiments. Storage at room temperature is only recommended for a short period of time (24 hours or less).

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