

# Reagents for cell detachment

## Instruction manual

Product	Size	Catalog number
Trypsin/EDTA (ready-to-use)	30 ml	C-41000
	125 ml	C-41010
	250 ml	C-41020
Trypsin Neutralizing Solution (0.05% (w/v) Trypsin Inhibitor in 0.1% (w/v) BSA)	30 ml	C-41100
	125 ml	C-41110
	250 ml	C-41120

### Product description

Trypsin is still the most widely employed enzyme used for cell detachment. Our Trypsin/EDTA (ready-to-use) solution exhibits standardized enzyme activity of 700 BAEE-U/ml and contains trypsin from extra-pure lots. This activity level of 700 BAEE-U/ml is functionally equivalent to a 0.04% solution. The EDTA concentration is 0.03% (w/v).

### Storage and stability

Store at -20°C in the dark immediately after arrival for long term storage. The product

is shipped with cool packs, so may arrive thawed and can be refrozen without any loss of quality. However, avoid repeated freeze/thaw cycles. Thawed solutions can be stored at 4 to 8°C and used for up to 6 weeks. If stored properly, the product is stable until the expiration date stated on the label.

### Quality control

All lots of PromoCell Cell Detachment Reagents are subjected to comprehensive quality control tests. Each lot is routinely tested for biological function, absence of cytotoxicity, and physical parameters such as

osmolality and pH level. Approved in-house lots are used as a reference.

In addition, all lots have been tested for the absence of microbial contaminants (fungi and bacteria).

### Intended use

The products are for *in vitro* research use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

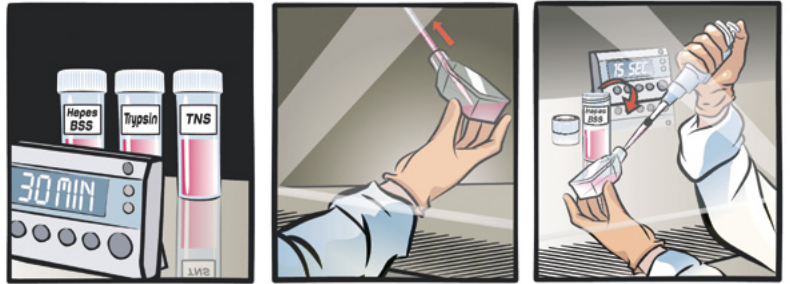
# Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

## Prepare the reagents and wash the cells

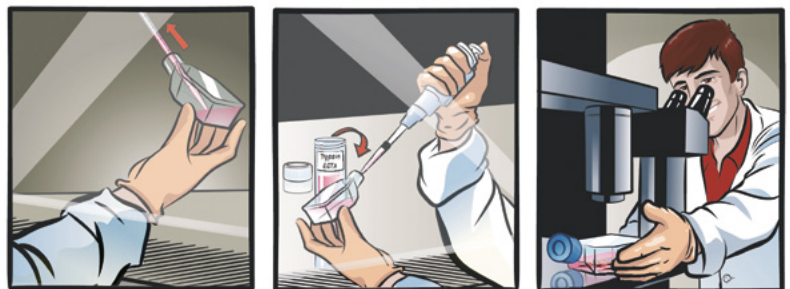
Place Trypsin/EDTA (ready-to-use) solution and Trypsin Neutralizing Solution at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100  $\mu$ l HEPES BSS Solution per  $\text{cm}^2$  of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

## Detach the cells

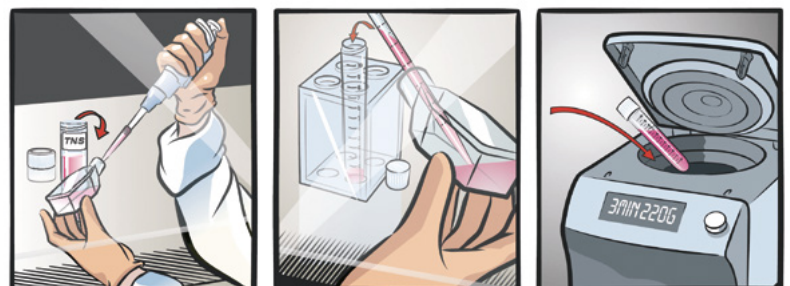
Carefully aspirate the HEPES BSS from the culture vessel. Add 100  $\mu$ l Trypsin/EDTA (ready-to-use) solution per  $\text{cm}^2$  of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen remaining cells.



3

## Neutralize the trypsin and harvest the cells

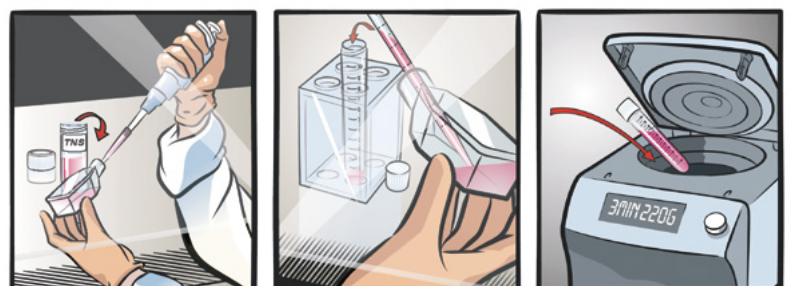
Add 100  $\mu$ l Trypsin/EDTA (ready-to-use) solution per  $\text{cm}^2$  of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



4

## Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium (step 2), and re-suspend the cells by carefully pipetting up and down. Determine the cell number and plate the cells according to the recommended seeding density in new cell culture vessels containing PromoCell Cell Growth Medium pre-warmed to 37°C. Place the vessels in an incubator (37°C, 5%  $\text{CO}_2$ ).



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