

# Cellvento® 4CHO-C Cloning Medium

## Chemically defined medium for single cell cloning

### Product description

Cellvento® 4CHO-C Cloning Medium is a chemically defined, animal component free medium developed for Single Cell Cloning (SCC) and stable pool selection and recovery of Chinese Hamster Ovary (CHO) cell lines. This new medium supports CHO and CHOZN® cell line development and can be used to replace EX-CELL® CHO Cloning Medium within the CHOZN® Technical Bulletin. It completes chemically defined media solutions for the entire process from cell line development to production scale.

### Application

The formulation is designed without hydrolysates or components of unknown composition and has superior performance and consistency between lots. The medium contains hypoxanthine and thymidine (HT) but for more selection flexibility it does not contain L-glutamine, Methionine sulfoximine (MSX), or Methotrexate (MTX).

### Cellvento® 4CHO-C Cloning medium For Single Cell Cloning

1. Serially dilute a stable pool culture to 0.5–1 cell/well in 200 µL to ensure a high probability of clonality. Serial dilutions can be performed using growth medium (i.e. EX-CELL® CD CHO Fusion), until the final dilution. The final dilution should be performed using the plating medium, a mix of 80% cloning medium and 20% conditioned medium. Recommended plates are sterile, clear 96 well, tissue culture treated, flat bottomed microplates.
2. Place the plates in the incubator at normal parameters. For clonal screening, minimize agitation.
3. Track clonality by using a microscope on Day 6 or 7 and Day 14. Culture may be supplemented with growth medium (i.e. EX-CELL® CD CHO Fusion), to account for evaporated medium.
4. Once confluent, clones can be consolidated to fewer plates and undergo further selection.

### For Stable Pool Selection and Recovery:

1. Plate stable pool at 5000 cells/well in 80% Cellvento® 4CHO-C Cloning Medium and 20% growth medium (i.e. EX-CELL® CD CHO Fusion).
2. Once confluent, passage into 2 plates by diluting 1:5. One will be used for propagation, and the other will be used for screening.

### Culture Techniques:

Minimize carryover of growth medium into single cell cloning plates to approximately 2% for optimal growth. Growth medium carryover may inhibit growth at such low seeding densities.

During dilution and screening, ensure supplemental growth medium is void of components that may interfere with selection.

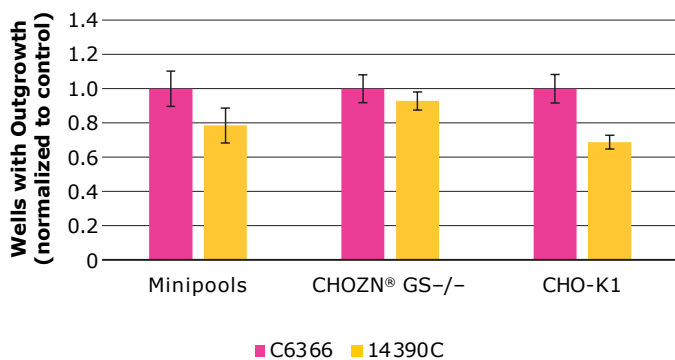
### Storage

Liquid medium should be stored at 2–8 °C protected from light.

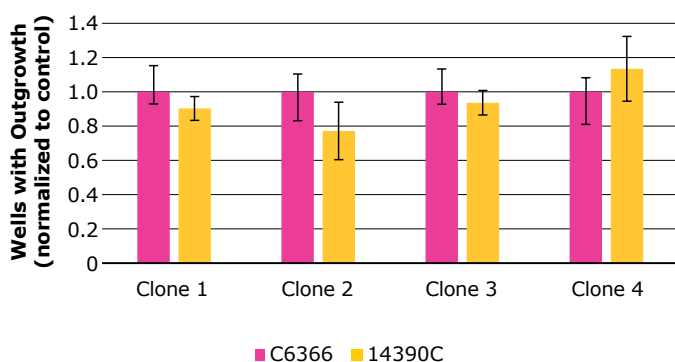
Do not use after expiration date.

### Comparability Study of Cellvento® 4CHO-C Cloning Medium (14390C) with EX-CELL® CHO CD Cloning Medium (C6366):

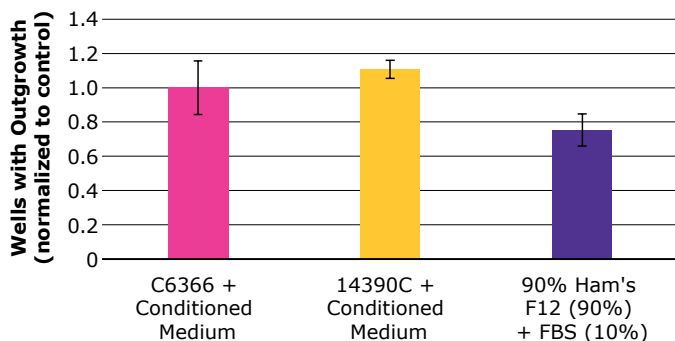
For each experiment, the number of wells with outgrowth was counted across all plates. An average was calculated for the control plates (C6366) and each experimental plate count was normalized to that average. An average of the normalized values, along with standard error of the mean (SEM), is shown in Figures 1–4.



**Figure 1:** Clone outgrowth was comparable between C6366 and 14390C. Cellvento® 4CHO-C performed more consistently across cell lines and pools.



**Figure 2:** Clone outgrowth was comparable between C6366 and 14390C across several clones.

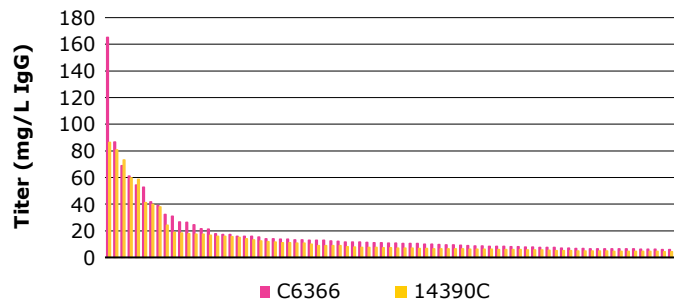


**Figure 3:** Clone outgrowth in the presence of conditioned medium was comparable between C6366 and 14390C. In the presence of 20% conditioned medium, Cellvento® 4CHO-C out performed Ham's F12 with 10% fetal bovine serum (FBS).

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**Figure 4:** Minipool selection had comparable titer profiles between C6366 and 14390C.

## Ordering Information

The medium is available in liquid format:

Catalogue Number	Product Name	Pack size
14390C-500mL	Cellvento® 4CHO-C Cloning Medium	500 mL

Ordering information for basal media options:

Catalogue Number	Product Name	Pack size
14365C-1000mL	EX-CELL® CD CHO Fusion	1000 mL

Ordering information for cell culture additives:

Catalogue Number	Product Name	Pack size
59202C-100mL	L-Glutamine solution	100 mL

CHOZN®  
Technical  
Bulletin



MilliporeSigma  
400 Summit Drive  
Burlington, MA 01803

