



## COMPONENT

**D-0710-1L5**

Korz Feed Stock

1000 ml

## STORAGE CONDITIONS

For long term storage, store the Korz Feed Stock at 4-12°C and protected from light. For short term storage the Feed Stock may be stored on the bench top at room temp for several days.

## BACKGROUND

To simplify the use of minimal medium for fed-batch fermentations, Scarab Genomics offers medium for both the batch phase and feeding phase of fed-batch fermentations. The Korz Feed Stock supplies glucose, magnesium, iron, and trace elements for the feeding stage of Modified Korz Medium fed-batch fermentations.

Scarab's Clean Genome strains were specifically designed for the production of biotherapeutic protein and DNA. The "cleanest" medium to use for biotherapeutic production is a chemically defined, minimal medium. Accordingly, Modified Korz Minimal Medium has been extensively tested with the Scarab Clean Genome Strains to verify its ability to support cell growth and the production of recombinant protein. Korz minimal medium is designed for high density fed-batch fermentation of *E. coli* (Korz et al. 1995, Sharma et al. 2007). The medium consists of phosphate buffer, magnesium, ferric citrate, trace elements, and uses glucose as the carbon source. The same base medium used for optimizing expression in shake flasks can also be used for fed-batch fermentation, thereby providing continuity between the two processes.

## BEFORE YOU BEGIN

- If using Modified Korz Minimal Medium with the Scarab Clean Genome® strains, these strains do not remain viable for extended periods (greater than 2 weeks) when stored at 4°C. We recommend preparing glycerol stock cultures of clones and storing at -80°C, or keeping plates at room temperature for up to 5 days.
- For protein expression, Scarab Hosts perform best at temperatures  $\geq 25^\circ\text{C}$ .
- To ensure that the cells grow on minimal media and to prevent a significant lag when transferring to liquid culture, streak from glycerol stocks onto minimal plates with 0.2% glucose and grow at 37°C for 24 h, at 30°C or 48 h, or at room temperature (RT) for several days (e.g., over the weekend). Colonies picked from these plates are used for cultures.
- Scarab's Clean Genome strains do not have flagella and tend to aggregate and drop fairly quickly from solution. To obtain accurate OD readings, cells should be mixed just before taking an aliquot for dilution, and dilution samples should be mixed just before taking an OD reading.



Korz Feed Stock  
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**FOR RESEARCH USE ONLY**

### USE OF KORZ FEED STOCK

The Korz Feed Stock is formulated to serve as the feed medium for the feeding stage of a fed-batch fermentation. Using sterile technique, transfer or connect the Korz Feed Stock to the fermentation device. Scarab recommends an exponential feed rate to control the growth rate to a constant value (Akesson et al. 1999; DeLisa et al. 1999). An initial feed rate for the fermentation is set to control the specific growth rate to 0.25 1/h. Glucose concentration in the fermenter is then confirmed to be zero periodically. If the glucose level rises above 2 g/L, shut off the feed pump until a DO spike is observed indicating glucose depletion. Then switch the feed pump on at a slightly reduced rate in order to prevent overfeeding again. When using an inducer to initiate or maintain expression, add the appropriate amount of inducer to the feed medium to maintain a constant concentration during the Fed-batch stage.

### REFERENCES

1. Korz DJ, Rinas U, Hellmuth K, Sanders EA, Deckwer WD. J Biotechnol. 1995 Feb 21;39(1):59-65. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*.
2. Sharma SS, Campbell JW, Frisch D, Blattner FR, Harcum SW. Biotechnol Bioeng. 2007 Dec 1;98(5):1056-70. Expression of Two Recombinant Chloramphenicol Acetyltransferase Variants in Highly Reduced Genome *Escherichia coli* Strains.
3. Akesson M, Karlsson EN, Hagander P, Axelsson JP, Tocaj A. Biotechnol Bioeng. 1999 Sep 5;64(5):590-8. On-line detection of acetate formation in *Escherichia coli* cultures using dissolved oxygen responses to feed transients.
4. DeLisa MP, Li J, Rao G, Weigand WA, Bentley WE. Biotechnol Bioeng. 1999 Oct 5;65(1):54-64. Monitoring GFP-operon fusion protein expression during high cell density cultivation of *Escherichia coli* using an on-line optical sensor.

### TROUBLESHOOTING

| Problem              | Possible Solution  |
|----------------------|--|
| No growth of culture | 1. Incorrect drug selection or drug concentration. Verify that proper concentration of antibiotic was added.<br>2. Strain being cultured is an auxotroph. Modified Korz Minimal Medium will not support the growth of an auxotrophic strain unless the appropriate supplement is added to the medium. Note that none of the Clean Genome strains are auxotrophs. |



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