



SCARAB GENOMICS LLC

CLEAN GENOME® *E. coli*

10X Modified Korz Medium with 0.2%
Glucose Kit
(Cat. No. D-0710-1002K, D-0710-1L2K)
FOR RESEARCH USE ONLY

COMPONENTS

	D-0710-1002K	D-0710-1L2K
10X Modified Korz Medium	100 ml (D-0710-100)	1000 ml (D-0710-1L)
50X Magnesium Sulfate	20 ml (D-0710-100M)	200 ml (D-0710-1LM)
50% Glucose	4 ml (D0710-100G)	40 ml (D-0710-1LG)

STORAGE CONDITIONS

Store the 10X Modified Korz Medium component at 4-12°C and protected from light. Working stocks of diluted 1X medium may be stored on the bench top at room temp for several days.

BACKGROUND

To simplify the use of minimal medium, Scarab Genomics offers a three component kit consisting of 10X Modified Korz Medium with separate 50X Magnesium Sulfate and 50% Glucose solutions. The concentrated minimal medium and associated Magnesium Sulfate and Glucose solutions are diluted to create a 1X medium. The diluted 1X medium containing Magnesium Sulfate and 0.2% glucose (and appropriate antibiotics) is used for expression optimization in shake flasks. The same medium (supplemented with additional glucose to a final concentration of 0.5% and additional phosphate buffer) also serves as the “batch” phase medium in 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 was originally designed for high density fed-batch fermentation of *E. coli* (Korz et al. 1995). 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. In fed-batch fermentations, the same medium is supplemented with higher glucose and phosphate buffer content. A separate Korz Feed Medium (Cat. No. D-0710-1L5) supplies glucose, magnesium, iron, and trace elements for the feeding stage of fed-batch fermentation.

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 or Korts 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.



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- 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.

PREPARATION OF 1X MODIFIED KORZ MEDIUM + 0.2% GLUCOSE

Create the 1X Modified Korz Medium by performing the following dilutions to produce 1 liter of 1X medium. Scale up or down as needed to produce other volumes of medium. The following protocol requires the use of sterile technique.

1. Transfer 100 mls of 10X Modified Korz Medium to 876 mls of sterile water.
2. Transfer 4 mls of 50% Glucose to the diluted Korz mixture.
3. Transfer 20 mls of 50X Magnesium Sulfate Solution to the diluted Korz mixture.
4. Add antibiotics as appropriate.
5. Aliquot as needed.

PREPARATION OF 1X MODIFIED KORZ MEDIUM + 0.2% GLUCOSE PLATES

1. Prepare agar plates using standard procedures.
2. Add 13 grams of bacto-agar to 863 mls of Milli-Q water and autoclave for 20 minutes.
3. Allow the solution to cool to approximately 50-60°C and add
 - 100 mls of 10X Modified Korz Medium
 - 4 mls of 50% Glucose
 - 20 mls of 50X MgSO₄
 - 1 ml of 1000X concentrated antibiotic stock as appropriate.

Mix thoroughly and pour plates.

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.

TROUBLESHOOTING

Problem	Possible Solution
No growth of culture	<ol style="list-style-type: none"> 1. Incorrect drug selection or drug concentration. Verify that proper concentration of antibiotic was added. 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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