10X Modified Korz Medium Kit
(Cat. No. D-0710-100K, D-0710-1LK)

FOR RESEARCH USE ONLY

COMPONENTS	D-0710-100K	D-0710-1LK
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)

STORAGE CONDITIONS

Store the 10X Modified Korz Medium component at 4-12ºC 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 two component kit consisting of 10X Modified Korz Medium and a separate 50X Magnesium Sulfate solution. The concentrated minimal medium and associated Magnesium Sulfate solution are diluted to create a 1X medium. A carbon source must then be added to support cell growth. We would recommend the equivalent of 0.2% glucose. The diluted 1X medium (with carbon source and appropriate antibiotics) is used for expression optimization in shake flasks. The same medium can also serves as the "batch" phase medium in fed-batch fermentations. Typically, the level of the carbon source would be adjusted to a higher level than that used in the shake flask e.g. when using glucose, the glucose level would be increased to 0.5%.

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. The user needs to supply 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 simply supplemented with higher carbon source content.

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 $\geq$ 25ºC.
- 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.

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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

Create the 1X Modified Korx Medium + carbon source by performing the following dilutions. The following protocol requires the use of sterile technique.

1. Transfer 1/10th volume of 10X Modified Korz Medium to the appropriate amount of sterile water.
2. Transfer 1/50th the volume of 50X Magnesium Sulfate Solution to the diluted Korz mixture.
3. Transfer the appropriate volume of carbon source (we recommend the equivalent of 0.2% glucose).
4. Add antibiotics as appropriate.
5. Aliquot as needed.

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	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. 3. No carbon source was added. Add the desired carbon source to the medium prior to use.

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