

# A Cell Culture Model for Producing High Titer Hepatitis E Virus Stocks

 Toni Luise Meister<sup>\*1</sup>, Mara Klöhn<sup>\*1</sup>, Eike Steinmann<sup>1</sup>, Daniel Todt<sup>1</sup>
<sup>1</sup>Molecular and Medical Virology, Ruhr University Bochum

\*These authors contributed equally

## Corresponding Author

 Daniel Todt  
 daniel.todt@ruhr-uni-bochum.de

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jove.com/video/61373

## Materials

Name	Company	Catalog Number	Comments
0.45 µm mesh	Sarstedt	83.1826	Harvest extracellular Virus
4 % Histofix	CarlRoth	P087.4	Immunofluorescence
Acetic acid	CarlRoth	6755.1	Collagen working solution
Amicon Ultra-15	Merck Millipore	UFC910024	Virus harvesting
Ampicillin	Sigma-Aldrich	A1593	Selection of transformed bacteria
ATP	Roche	11140965001	<i>in vitro</i> transcription and electroporation
BioRender	BioRender		Figure Generation
CaCl <sub>2</sub>	Roth	5239.2	Cytomix
Collagen R solution 0.4 % sterile	Serva	47256.01	Collagen working solution
CTP	Roche	11140922001	<i>in vitro</i> transcription
Cuvette	Biorad	165-2088	Electroporation
DAPI	Invitrogen	D21490	Immunofluorescence
DMEM	gibco	41965-039	Cell culture
DNase	Promega	M6101	<i>in vitro</i> transcription
DTT	Promega	included in P2077	<i>in vitro</i> transcription
EGTA	Roth	3054.3	Cytomix
<i>Escherichia coli</i> JM109	Promega	L2005	Transformation
Fetal bovine serum	gibco	10270106	Cell culture
Fluoromount	SouthernBiotech	0100-01	Immunofluorescence
GenePulser Xcell Electroporation System	BioRad	1652660	Electroporation
Gentamycin	gibco	15710049	Cell culture
GTP	Roche	11140957001	<i>in vitro</i> transcription
H <sub>2</sub> O	Braun	184238001	Immunofluorescence
Hepes	Invitrogen	15630-03	Cytomix
Horse serum	gibco	16050122	Immunofluorescence

K <sub>2</sub> HPO <sub>4</sub>	Roth	P749.1	Cytomix
KCL	Roth	6781.3	Cytomix
KH <sub>2</sub> PO <sub>4</sub>	Roth	3904.2	Cytomix
L-Glutamin	gibco	25030081	Cell culture
L-Glutathione reduced	Sigma-Aldrich	G4251-5G	Cytomix
MEM	gibco	31095-029	Cell culture
MEM NEAA (100×)	gibco	11140-035	Cell culture
MgCl <sub>2</sub>	Roth	2189.2	Cytomix
Microvolume UV-Vis spectrophotometer NanoDrop One	Thermo Fisher	ND-ONE-W	DNA/RNA concentration
MluI enzyme	NEB	R0198L	Linearization
NEB buffer	NEB	included in R0198L	Linearization
NucleoSpin Plasmid kit	Macherey & Nagel	740588.250	Plasmid preparation
NucleoSpin RNA Clean-up Kit	Macherey & Nagel	740948.250	RNA purification
PBS	gibco	70011051	Cell culture
Pen/Strep	Thermo Fisher	15140122	Cell culture
Plasmid encoding full-length HEV genome (p6_G1634R)	Todt et.al		Virus production
Plasmid encoding full-length HEV genome (p6_WT)	Shukla et al.	GenBank accession no. JQ679013	Virus production
Primary antibody 1E6	LS-Bio	C67675	Immunofluorescence
Primary antibody 8282			Rainer Ulrich, Friedrich Loeffler Institute, Germany
QIAprep Spin Miniprep Kit	Qiagen	27106	DNA extraction
Ribo m <sup>7</sup> G Cap Analog	Promega	P1711	<i>in vitro</i> transcription
RNase away	CarlRoth	A998.3	RNA purification
RNasin (RNase inhibitor)	Promega	N2515	<i>in vitro</i> transcription
Secondary antibody donkey anti-mouse 488	Thermo Fisher	A-21202	Immunofluorescence
Secondary antibody goat anti-rabbit 488	Thermo Fisher	A-11008	Immunofluorescence
Sodium Pyruvat	gibco	11360070	Cell culture
T <sub>7</sub> RNA polymerase	Promega	P2077	<i>in vitro</i> transcription
Transcription Buffer	Promega	included in P2077	<i>in vitro</i> transcription
Triton X-100	CarlRoth	3051.3	Immunofluorescence
Trypsin-EDTA (0.5 %)	gibco	15400054	Cell culture
ultra-low IgG	gibco	1921005PJ	Cell culture
UTP	Roche	11140949001	<i>in vitro</i> transcription