1555. Medium for tick cells (Rickettsia)

Cultivation of IRE11 cells for infection with *Rickettsia monacensis*

L15C (Modified Leibovitzs' L15) used to culture host cells (IRE11). L15C medium is a modification of L15B medium, previously described in detail (Munderloh UG, Kurtti TJ. 1989. Formulation of medium for tick cell culture. Exp. Appl. Acarol. 7:219–29).

Alterations were made to the following reagents:

L-aspartic acid	0.4485 g/L
L-glutamine	0.5 g/L
L-proline	0.45 g/L
L-glutamic acid	0.25 g/L
α–ketoglutaric acid	0.4485 g/L
D-glucose	18.018 g/L
NaOH	5 mM

Prior to use in supplemented medium, L15C was diluted by the addition of 33 % water by volume.

Supplemented L15C medium is used with

Foetal Bovine Serum	10 %
Tryptose Phosphate Broth	5%
lipoprotein concentrate (LPC; MPBiomedical)	0.1 %
HEPES	25 mM
$NaHCO_3$	0.25 %

The pH is adjusted to pH 7.5 using 1 M NaOH.

This medium and the cultivation of tick cells is described in the "**Basic Tick Cell Culture Methods**" provided as pdf-file (see strain entry for DSM 29017: cultivation conditions) by Tim Kurtti & Uli Munderloh, University of Minnesota.

Infection with *Rickettsia monacensis* or *R. buchneri*:
One 25 cm^2 flask of well-grown cells is infected with 1 ml of the Rickettsia strain (quickly thawed from -80°C) and centrifuged for 1 h onto the cell layer at 1600 rpm at 20°C. Incubate at 30°C with closed lid (without CO_2) and look for bacteria and destroyed cells after 4-6 days.