

## 403: BSK-MEDIUM

This recipe contains strain-specific modifications for *Borrelia burgdorferi* DSM 4681 \*

Final volume: 1429 ml

|                   |         |    |
|-------------------|---------|----|
| <b>Solution A</b> | 1000.00 | ml |
| <b>Solution B</b> | 200.00  | ml |
| <b>Solution C</b> | 143.00  | ml |
| <b>Solution D</b> | 86.00   | ml |

1. Combine the warm (37°C) solutions A, B, C, D, and filter-sterilize the warm medium. Solution B can also be added after filtration.
2. Alternatively, buy supplemented or un-supplemented BSK-H medium (Fa. Bio&SELL or Sigma) and supplement the medium according to manufacturer's advice. Sterilize the mixed and warm (37°C) medium by filtration.

\* Microaerophilic (candle jar)

### Solution A

|                                            |        |    |
|--------------------------------------------|--------|----|
| CMRL 1066 (GIBCO 042-1545, glutamin 10 x)  | 100.00 | ml |
| Proteose peptone no. 2 (DIFCO 0121-01-3)   | 5.00   | g  |
| Tryptone peptone (DIFCO 0123-01)           | 1.00   | g  |
| Proteose yeastolate (TC) (DIFCO 5577-15)   | 1.00   | g  |
| HEPES buffer (acid) (SIGMA H3375)          | 6.00   | g  |
| Sodium citrate                             | 0.70   | g  |
| Glucose (alpha-, D+)                       | 3.00   | g  |
| Na-pyruvate                                | 0.80   | g  |
| N-Acetylglucosamine                        | 0.40   | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O     | 0.30   | g  |
| L-Glutamine                                | 0.10   | g  |
| Double distilled water (filter-sterilized) | 900.00 | ml |

Stir slowly at 4 - 10°C for 3 hours, adjust pH to 7.6 with 5 M NaOH. Filter sterilize.

### Solution B

|                        |        |    |
|------------------------|--------|----|
| Gelatine               | 14.00  | g  |
| Double distilled water | 200.00 | ml |

Dissolve 14.0 g gelatine in 200 ml bidest. water, autoclave 15 minutes at 115°C.

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### Solution C

|                                       |        |    |
|---------------------------------------|--------|----|
| Bovine serum albumin (Sigma No A9647) | 50.05  | g  |
| Double distilled water                | 143.00 | ml |

Dissolve 50.05 g bovine serum albumine, fract. V (important: Sigma No A9647) in 143 ml bidest. water, or use BSA-solution Sigma A7409.

### Solution D

|                                              |       |    |
|----------------------------------------------|-------|----|
| Rabbit serum (inactivated, GIBCO 037-06120M) | 86.00 | ml |
|----------------------------------------------|-------|----|