

# BD Select™ CD1000 Medium

## Hydrolysate Usage Protocol

### Preparation of a 10% Hydrolysate Stock Solution (100 g/L)

1. Add 50 mL of WFI (or equivalent) to a mixing vessel
2. Weigh 10.00 g of hydrolysate and add to the vessel while stirring
3. Allow the hydrolysate to stir until all particles are dissolved and solution has cleared
4. QS the hydrolysate mixture by pouring the solution into a 100 mL volumetric flask and adding water to the graduation mark
5. Sterile filter the hydrolysate mixture and store at 2-8°C



### Hydrolysate Titration

Hydrolysate titration should be performed in 125 mL shake flasks in duplicates. It is recommended to use a concentration range from 0-13 g/L. Add all cell specific supplements to media before starting assay (e.g. L-Glutamine, HT etc.). Run assay for a minimum of 14 days or until cell viability falls below 50%, whichever occurs first.

1. Add no hydrolysate to 50 mL of base media for 0 g/L hydrolysate concentration
2. Add 500 µL of 10% hydrolysate solution to 50 mL of base media for 1 g/L hydrolysate concentration
3. Add 1.5 mL of 10% hydrolysate solution to 48.5 mL of base media for 3 g/L hydrolysate concentration
4. Add 2.5 mL of 10% hydrolysate solution to 47.5 mL of base media for 5 g/L hydrolysate concentration
5. Add 3.5 mL of 10% hydrolysate solution to 46.5 mL of base media for 7 g/L hydrolysate concentration
6. Add 4.5 mL of 10% hydrolysate solution to 45.5 mL of base media for 9 g/L hydrolysate concentration
7. Add 5.5 mL of 10% hydrolysate solution to 44.5 mL of base media for 11 g/L hydrolysate concentration
8. Add 6.5 mL of 10% hydrolysate solution to 43.5 mL of base media for 13 g/L hydrolysate concentration

### Mixture Design

Hydrolysate blends can have synergistic effects in some base media, so there is a significant benefit to evaluating both individual and blended hydrolysates. A mixture design is an easy way to begin this evaluation. To perform this type of evaluation, it is necessary to determine the maximum concentration of hydrolysate to be added to the culture. Then, the two hydrolysates should be combined in a manner to achieve the total hydrolysate concentration that is acceptable.

| Condition  | Hydrolysate #1 | Hydrolysate #2 |
|------------|----------------|----------------|
| Mixture #1 | 100%           | 0%             |
| Mixture #2 | 75%            | 25%            |
| Mixture #3 | 50%            | 50%            |
| Mixture #4 | 25%            | 75%            |
| Mixture #5 | 0%             | 100%           |

| Condition  | Hydrolysate #1 | Hydrolysate #2 | Total Hydrolysate Concentration |
|------------|----------------|----------------|---------------------------------|
| Mixture #1 | 6 g/L          | 0 g/L          | 6 g/L                           |
| Mixture #2 | 4.5 g/L        | 1.5 g/L        | 6 g/L                           |
| Mixture #3 | 3 g/L          | 3 g/L          | 6 g/L                           |
| Mixture #4 | 1.5 g/L        | 4.5 g/L        | 6 g/L                           |
| Mixture #5 | 0 g/L          | 6 g/L          | 6 g/L                           |

**Example: Total hydrolysate concentration = 6 g/L**

### Endpoint Assay

Measure cell viability, cell density and protein production periodically throughout the culture and on final day of culture to identify the optimal concentration for hydrolysate supplementation.

For more information, including technical adaptation protocols, visit us today at [bdbiosciences.com/advbio](http://bdbiosciences.com/advbio), call BD Technical Service & Support at 800.638.8663 or contact your Bioprocess Application Specialist.



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