HY01

Version 1.0

HY01 is a basal medium with balanced nutrition and can support high-density growth and stable expression of hybridoma.

Product	Catalog No.	HT	L-Gln	D-Gluc	Growth Factors	Hydrolysates	Proteins
HY01	22318-1429	Yes	Yes	3 g/L	No	No	No
CD 293 FA	99151-1241	No	Yes	60 g/L	No	No	No
CD 293 FB	99035-1242	No	No	No	No	No	No

Intended Use

For research and further manufacturing use only.

Storage and Stability

The dry powder medium should be stored at 2–8°C and protected from light. The product is stable for 24 months, when unopened and stored properly. This product is very hygroscopic and should be kept in a dry environment away from moisture. **Supplementation of 2–4 mM glutamine into liquid HY01 medium stored for over 3 months is recommended.**

Prepare Liquid Medium from Dry Powder

HY01

1. Fill the mixing container with purified water (20–30°C) at 90% of the final volume.
2. Slowly add 20.0 g/L of dry powder medium with gentle stirring. Mix for 10 minutes until there is no dry powder on the solution.
3. Adjust the pH to 6.7–7.0 using 1 M–5 M NaOH or HCl. Mix for 10 minutes until dissolving completely.
4. Add 2.2 g/L of sodium bicarbonate. Mix for 10 minutes.
5. Adjust the final volume with purified water. Mix for 5 minutes.
6. Filter the media using a membrane filter with 0.22 μm pore size immediately, then store at 2–8°C.

CD 293 FA

1. Measure 80% of final volume purified water at room temperature (20–30°C).
2. Add 180.10 g/L dry powder, mix for a minimum of 30 minutes.
3. Adjust pH to 6.5–6.9 with a 1.0N–5.0N NaOH solution, mix for 30 minutes.
4. Dilute to the final volume with purified water, mix for 10 minutes.
5. Sterilize the medium immediately using a 0.22 μm membrane filter.

CD 293 FB

1. Measure 80% of the final volume purified water at room temperature(20–30°C).
2. Add 94.97 g/L dry powder to water, mix for 30 minutes.
3. Adjust pH to 10.8–11.4 with a 1.0N–5.0N NaOH solution, mix for 30 minutes.

4. Dilute to the final volume with purified water, mix for 10 minutes.
5. Sterilize the medium immediately using a 0.22 μm membrane filter.

Adapt hybridoma to HY01

We recommend using the following steps to adapt cells in serum media to HY01.

1. Culture cells in HY01 with 5% serum for 48 h by seeding at 0.3 × 10^6 cells/mL. When the viability is greater than 95% and the doubling time in the medium is not longer than that in the previous medium, the culture is considered to have been adapted to the new medium.
2. Culture cells in HY01 with 2% serum for 48 h by seeding at 0.3 × 10^6 cells/mL. When the viability is greater than 95% and the doubling time in the medium is no longer than that in the previous medium, the culture is considered to have been adapted to the new medium.
3. Culture cells in 100% HY01 for 48 h by seeding at 0.3 × 10^6 cells/mL. When the viability is greater than 95% and the doubling time in the medium is no longer than that in the previous medium, the culture is considered to have been adapted to HY01.

Protein Expression

We recommend using methods as follow to culture hybridoma and express protein of interest.

Basal Medium	Application	Note	Feed 1	Feed 2
HY01	Biopharmaceutical, IVD	Basal medium concludes 4 mM glutamine	CD 293 FA	CD 293 FB
Culture conditions	Seeding density: 0.5–1 × 10^6 cells/mL; Culture temperature: 36.5–37.0°C，pH:7.1 ± 0.2，DO：40%。（Rotational Speed: 120 rpm; Shaking throw: 50 mm; 5–8% CO_2 ）			
Feed process	● Feed for the first time at 48 h or when cell density reaches 2.5 × 10^6 cells/mL：3% Feed 1 + 0.3% Feed 2 + 4 mM glutamine; maintain the concentration of glucose at 4 g/L. ● Feed for the second time at 96 h：3% Feed 1 + 0.3% Feed 2 + 4 mM glutamine; maintain the concentration of glucose at 4 g/L. ● End culture when the cell density is less than 50%.			