

Seahorse Recipes

XF Assay Media (unbuffered, glucose free, pyruvate free)

Available from Sigma (Sigma D5030-1L) or Seahorse (Part #100965-000)

Seahorse XF Assay Media is specially formulated for XF assays. Just add the required amount of glucose, Glutamax, and Sodium Pyruvate and adjust the pH.

Unbuffered DMEM (Assay DMEM)

A. Reagents:

- Dulbecco's Modified Eagle's Medium Base 8.3g/L (Seahorse Part #100965-000; Sigma D5030-1L)
- 100x (200mM) GlutaMax-1 (Gibco 35050-061)
- 100mM Sodium Pyruvate (Sigma S8636) (Assay Dependent)
- Glucose (Sigma G8270) (Assay Dependent)
- NaCl (Sigma S3014)
- Phenol Red (Sigma P-5530)

For Sigma DMEM:

Dissolve DMEM Base in 500 mL dH₂O. Separately, dissolve 1.85g NaCl in 500 mL dH₂O. Combine NaCl solution with DMEM Base solution. Add 15 mg Phenol Red.

For Sigma and Seahorse DMEM:

Remove 20 mL from the media. Add 10mL 100mM Sodium Pyruvate (assay dependent). Add glucose powder for desired concentration (4.5g = 25mM glucose). Warm media to 37°C, then pH to 7.4 using 5M NaOH (Sigma). Filter sterilize. Media can be stored at 4°C for later use.

Compunds:

FCCP:

Prepare a 30 mM solution of FCCP in DMSO. Dilute the 30 mM stock to 300 uM in DMSO. These can be stored frozen. For injection, dilute the 300 uM stock to 3 uM in unbuffered DMEM. pH the 3 uM solution to 7.4. For one XF24 plate, you will need approximately 2 ml of 3 uM FCCP. The concentration loaded will be 3 uM. *The 3 uM FCCP will be injected during the assay and diluted 10x for a **final concentration of 300 nM**.*

Rotenone:

Prepare a 50 mM stock of rotenone in DMSO. Dilute the 50 mM stock to 1 mM with DMSO. Dilute the 1 mM stock to 100 uM in DMSO. These can be stored frozen. For injection, dilute the 100 uM stock to 1 uM in unbuffered DMEM. pH to 7.4. For one XF24 plate, you will need approximately 2 ml of 1 uM rotenone. *The 1 uM rotenone will be injected during the assay and diluted 10x for a **final concentration of 100 nM**.*

Oligomycin:

Prepare a 50 mM stock of oligomycin in DMSO. Dilute the 50 mM stock to 1 mM with DMSO. These can be stored frozen. For injection, dilute the 1 mM stock to 10 uM in unbuffered DMEM. pH to 7.4. For one XF24 plate, you will need approximately 2 ml of 10 uM oligomycin. *The 10 uM oligomycin will be injected during the assay and diluted 10x for a **final concentration of 1 uM**.*
