IFO50268
EHS

As described in our website, this cell line does not grow in culture (non cultivable).
EHS can grow only by the transplantation to the syngenic mice.
https://cellbank.nibiohn.go.jp/~cellbank/en/search_res_det.cgi?ID=1833

Subcutaneous transplantation to C57/BL6 mice will produce visible solid tumor 4 weeks after transplantation.

[The status of frozen EHS]

Before freezing the EHS tumors were suspended in Hanks' balanced salt solution and were broken into small tumor fragments by passing through once 10 mL syringe without needle and then through syringe with 19 G needle. Then the tissue fragments were frozen with freezing medium (10% DMSO, 20% FBS in Eagle's minimum essential medium).

[Thawing and transplantation]

1. After thawing frozen vial, transfer all vial contents to 10 mL Hanks' balanced salt solution (HBSS) or saline in 15 ml centrifugation tube.

2. To remove DMSO in freezing medium, spin down the tumor fragments at 1000 rpm for 3 min, and re-suspend the tumor fragments in small amount of HBSS (or saline).

3. The volume is not specified. Transplant the tumor fragments (by syringe or so) subcutaneously to the neck region. We used 10 weeks mice. Original method said that 4 x 10^6 cells/animal. However, cell count of tissue fragments was not possible. We believe that our vial contains much more number of cells. It will be possible to transplant to 2-3 mice(*) from one vial contents.

   (*)JCRB's recommendation is transplantation to 2 mice.

4. Usual subcutaneous transplantation to C57/BL6 mice will produce visible solid tumor 4 weeks after transplantation. Tumor size at end point will be 2-4 cm in diameter.

Notes:
1) Although our vial contains enough amount of EHS tissue fragments, these fragments tend to stick to the inside surface of centrifugation tubes and pipettes and may lost during pipetting and centrifugation. So please take care the recovery of tissue fragments during handling.

2) JCRB used 20 G needle for the injection of EHS tissue fragments to mice. However, the size of tissue fragment is relatively large, and sometimes some large fragments can not pass through the 20 G needle. So, please carefully check that the tissue fragments are certainly injected to mice.

The 18 G needle can pass large tissue fragments but JCRB did not use it due to the risk of leakage after the transplantation.

3) Our recommendation is to transplant to 2 mice from 1 vial contents.

JCRB's transplantation protocol detail.

1. To remove DMSO, the suspension of tissue fragments was transfer to 10 mL of HBSS (or saline) in 50 mL tube(*1).
2. Centrifuge at 300 g for 5 min.
3. Remove the supernatant.
4. Re-suspend the tissue fragments in 10 mL of HBSS (or saline)
5. Centrifuge at 300 g for 5 min.
6. After the second centrifugation, remove the supernatant thoroughly with pipettman tip.

   (*1) At the last step, the tissue fragments were aspirate by 2 mL syringe. So, use of 50 mL tube is adequate).
   (*2) The reason for 2 times washing and use of pipettman tip is to remove the DMSO thoroughly.

7. Re-suspend the tissue fragments to approximately 700 uL of saline. [The volume (700 uL) is larger than 200 uL x 2 mice (=400 uL), since the suspension is aspirated by syringes later.]

8. (JCRB used pipetteman tip to resuspend the tissue fragment to loosen the tissue pellet.)

9. Using 2 mL syringe x 2 with 18 G needle, aspirate the suspension of tissue fragments to 2 syringes (approx. 300 uL/syringe) (*3).

   (*3) The reason why to prepare 2 syringes is to transplant the tissue suspension to 2 mice. If only one syringe is used for transplantation to multiple mice, there is a risk for clogging of tissues in the needle. In addition, it may cause an uneven amount of transplantation to each mouse.

10. Transfer these syringes on ice(*4) to breeding room for experimental mice.

    (*4) JCRB's procedure is to stand the syringe in 50 mL tubes cooled with ice.

11. Change the needle to 20G
12. Inject approximately 200 uL of tissue fragment suspension to one mouse subcutaneously(*5).

    (*5) In some cases, the tissue fragments may be clogged in 20G needle. So, it is recommended to prepare 18G needle for the safe.