

## Total RNA – Murine Embryonic Stem Cell Line D3

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**CATALOG NUMBER:** CBA-304

**STORAGE:** -80°C; avoid freeze/thaw

**QUANTITY AND CONCENTRATION:** 50 µg at 1.0 mg/mL

**SHELF LIFE:** 6 months from date of receipt under proper storage conditions

### **Background**

Embryonic stem (ES) cells are continuous proliferating stem cell lines of embryonic origin first isolated from the inner cell mass (ICM). Two distinguishing features of these ES cells are their ability to be maintained indefinitely in an undifferentiated state and their potential to develop into any cell within the body.

ES-D3 was first isolated from day 4 129/Sv blastocysts in 1985 by Doetschmann et al. When injected into himeric blastocysts, ES-D3 contributed to the germ line. Alkaline phosphatase is routinely used as a marker of undifferentiated pluripotent stem cells. ES-D3 cells propagated on a feeder layer of irradiated STO mouse fibroblasts in the presence of recombinant mouse LIF are positive for alkaline phosphatase activity. Spontaneous differentiation of embryoid body cells can be induced by leukemia inhibitory factor withdrawal.

### **Quality Control**

1. Monitor ES alkaline phosphatase activity in a control D3 plate to ensure the undifferentiated stage of D3 cells before RNA isolation step.
2. The quality and purity of total RNA are tested to ensure the A260/280 ratio is between 1.8 and 2.0.
3. The integrity of total RNA is examined on denatured agarose gel for the presence of intact bands of 18S and 28S ribosomal RNA.

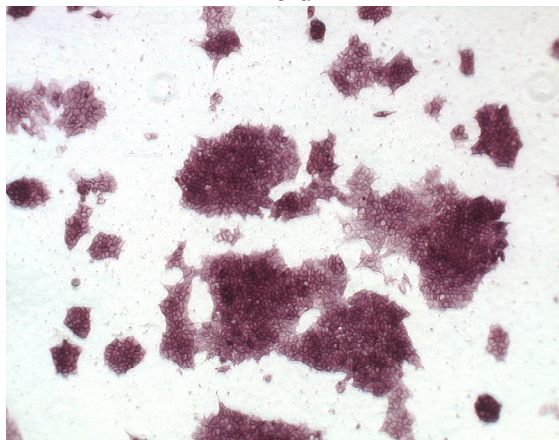
### **Methods**

1. Grow undifferentiated D3 cells on gelatin-coated dishes in the presence of LIF.
2. Before the RNA isolation step, examine ES alkaline phosphatase activity in a control plate using StemTAG™ Alkaline Phosphatase Staining Kit (#CBA-300).
3. Total RNA is isolated by RTIZOL method and dissolved in RNase-free water.
4. Determine RNA concentration by measuring OD 260 nm.

### **Example of Results**

The following figures demonstrate typical results. One should use the data below for reference only. This data should not be used to interpret actual results.

### 4X Field



**Figure 1. Alkaline Phosphatase Activity of Murine ES-D3 Cells.** AP activity on undifferentiated cells was determined using the StemTAG™ Alkaline Phosphatase Staining Kit (Cat. #CBA-300).

### References

1. Wobus AM, Holzhausen H, Jäkel P et al. (1984) *Exp Cell Res* 152:212–219.
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. (1998) *Science* 282:1145–1147.
3. Smith AG, Nichols J, Robertson M et al. (1992) *Dev Biol* 151:339–351.
4. Reubinoff BE, Pera MF, Fong CY et al. (2000) *Nat Biotechnol* 18:399–404.

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