Materials & Equipment
Cell lines

Following cell lines were used in the experimental investigations.

- Recombinant CHO cells with recombinant human EPO expressing gene (INDUS Biotherapeutics Ltd, Ahmedabad, India).
- TF-1 (Kitamura et al., 1989 and Kitamura et al., 1991), human erythroleukemia established from the bone marrow of a 35-year-old man with erythroleukemia (ECACC, European collection of cell cultures, Wiltshire, England, UK) for in vitro bioassay

Cell culture media

Basal medium

- DMEM for growth medium (GM) and DMEM/F12 for production medium (PM), RPMI 1640 (Sigma, Buchs SG, Switzerland/ Hyclone, South Logan, UT),

Serum

- FBS (Sigma, Buchs SG, Switzerland/ Hyclone, South Logan, UT).

Other media supplements

- Glutamine, Sodium bicarbonate, Sodium pyruvate, Glucose, Pluronic F-68 (Sigma, Buchs SG, Switzerland/ Hyclone, South Logan, UT
- NEAA’s (Invitrogen, Carlsbad, California)
- Insulin (Novo Nordisk India Private Ltd, Bangalore, India)
- GM-CSF (Zenotech Laboratories, Hyderabad, India)

Antibiotics

- Gentamycin (Ranbaxy, Haryana, India).
- Pencillin-Streptomycin (Sigma, Buchs SG, Switzerland/ Hyclone, South Logan, UT, USA).
Reagents

- Isopropyl alcohol (IPA), Hypochlorite solution, Acetonitrile, 0.1M HCl, 0.1M NaOH, 2-Proponol, Butanol, Glacial acetic acid, Sodium thiosulphate, Silver nitrate, Sodium carbonate, Methanol, Formaldehyde, Tris-HCl, SDS, Tetramethylene ethylenediamine (TEMED), Ammonium per-sulphate (APS), Tris-HCl, Glycine, Glycerol, Bromophenol blue, Acrylamide, Bis-acrylamide, Sodium acetate, Methanol, Butanol, TFA (Merck Specialties Private Limited, Navi Mumbai, India).

- Trypsin, Trypsin-EDTA, Di-sodium hydrogen phosphate (Na₂HPO₄), Sodium dihydrogen phosphate (NaH₂PO₄), Sodium dodecyl sulfate (SDS), DMSO, MTT, TEMED, Sodium azide, Coomassie Brilliant Blue R-250, N-Acetylnuraminic acid, Skimmed milk powder, Bromophenol blue (Sigma, Buchs SG, Switzerland).

- Sodium Phosphate dibasic anhydrous (Na₂PO₄), Potassium Phosphate monobasic (KH₂PO₄), Potassium Chloride (KCl), Sodium Chloride (NaCl), Sodium Hydroxide (NaOH), Hydrochloric Acid (HCl), Tris Base (C₄H₁₁NO₃), Tris amino-methane, Sodium Citrate dihydrate (C₆H₃Na₃O₇·2H₂O), Citric Acid anhydrous (C₆H₈O₇), Citric Acid anhydrous (C₆H₈O₇), Glacial Acetic acid (CH₃COOH), Sodium Acetate anhydrous (CH₃COONa), Isopropyl Alcohol (C₃H₈O) & Triton-X 100, ethanol, Tris base, Acetic acid, Tri Sodium citrate dehydrate, Citric acid, Orthophosphoric acid, Resorcinol, Copper sulfate, Butyl acetate, Urea, Trichloroacetic acid (J.T. Baker, Mumbai, India).

- Rabbit anti EPO antibody, Goat anti-rabbit ALP conjugate, 5- BCIP (5-Bromo-4-Chloro-3'-Indolylphosphate) and NBT (Nitro-Blue Tetrazolium Chloride). (Banglore Genei Pvt Ltd., Bangalore, India).

- Nitrocellulose membrane (Pall India Pvt. Ltd., Mumbai, India)

- Rabbit anti EPO antibody (R&D systems, Bangalore, India).

- N-Acetyl Nuraminic acid (Super Religare Laboratories, New Delhi, India).

- Resorcinol (Qualigens Fine Chemicals, Mumbai, India).

- Goat anti-rabbit ALP conjugated antibody (Banglore genei P Ltd., Bangalore, India).
• Blue Sepharose FF, SP Sepharose FF, Q Sepharose FF, Q Sepharose FF, Sephadex G 25, Q Sepharose FF Sephacryl S-200 (GE Healthcare, Chennai, India).

Consumables

- Tissue culture flasks, T-25/ T-75/ T-175 cm², Tissue culture plates-96 wells (Griener, GmbH, Germany)
- Roller bottles (BD Biosciences, San Jose, CA, USA)
- Serological pipettes- 2 mL, 5 mL, 10 mL, 50 mL (Griener, GmbH, Germany)
- Glass bottles- 250 mL, 1L, 2L (Schott Duran, GmbH, Germany)
- Cryovials- 2 ml (Griener, GmbH, Germany)
- Centrifuge tubes: 0.6 mL, 1.5 mL, 15 mL and 50 mL (Tarsor, North Syracuse, NY).
- Micro tips: 20 µL, 300 µL and 1mL (Axygen Scientific, Inc., Union City, CA, USA).
- Beakers: 1L, 2L (Tarsor, North Syracuse, NY)
- Filter Unit with 0.2 µm membrane filters (Pall India Pvt. Ltd., Mumbai, India).
- Gauze cloth (Kayco India, New Delhi, India).
- Liquid Nitrogen (LN₂) (Bhagath Enterprises, Ahmedabad, India)
- Syringe for repeat pipette: 1250 µL (Gilson, Inc., Middleton, WI, USA).
- 10 kDaPellicon membrane (Millipore, Billerica, MA, USA)
- PVDF membrane (Pall India Pvt. Ltd., Mumbai, India)
- Nitrocellulose membrane, nylon pads, glass rod, Whatman No.1 & 3 filter paper (Millipore, Billerica, MA, USA)

Reference material (Standards)

- rhEPO WHO Standard, NIBSC (South Mimms Potters Bar, Herts EN6 3QG, UK).
- Control standard, Eprex (Cilag AG, Schaffhausen, Switzerland).
- Molecular weight marker, range: 14.3 to 97.4 kda (Bio-Rad Laboratories, Hercules, CA, USA).
- Broad prestained SDS-PAGE standards, broad range (Bio-Rad Laboratories, Hercules, CA, USA)
- Broad pre-stained SDS-PAGE standards, broad range (Bio-Rad Laboratories, Hercules, CA, USA)
- Ampholytes: Pharmalyte, pH range: 2.5-5.0 & Pharmalyte, pH range: 3-9 (Bio-Rad Laboratories, Hercules, CA, USA)

**Equipment**

- CO₂ Incubator (Binder, Tuttlingen, Germany)
- Incubator 25°C (Binder, Tuttlingen, Germany)
- Orbital shaker, Horizontal shaker (Heidolph Instruments, GmbH, Germany)
- Class II Type A Biosafety Hood (ESCO Biotech Pvt. Ltd., Mumbai, India)
- Inverted Phase contrast microscope (Olympus Imaging America Inc., Center Valley, PA)
- Centrifuge (Sigma, Buchs SG, Switzerland)
- pH meter (Mettler-Toledo, Inc., Columbus, OH, USA)
- Spinner flaks (Wheaton Industries Inc., Millville, NJ, USA)
- Conical (Erlenmeyer) Flask (Wheaton Industries Inc., Millville, NJ, USA)
- Roller Incubator (wheaton Industries Inc., Millville, NJ, USA)
- Water Bath (Julabo, Seelbach, Germany)
- Liquid Nitrogen Can (CHART Industries Inc., Marietta, GA, USA)
- Cell Count Analyzer (Beckman Coulter India Pvt. Ltd., Mumbai, India)
- Micro-Stirrer (Wheaton Industries Inc., Millville, NJ, USA)
- Refrigerator: 2-8°C (Samsung Electronics (UK) Ltd, Chertsey, Surrey KT16 0PS); -20°C (Neubrunswick, NJ, USA) & –80°C freezer (Neubrunswick, NJ, USA)
- SDS-PAGE gel apparatus-Vertical Slab gel System (Atto Bioscience and Biotechnology, Tokyo, Japan).
- Weighing balance (Atlas Weighing Equipments, New Delhi, India).
- Cryocooler (Tarsons Product Pvt. Ltd., Kolkatta, India)
- RP-HPLC System (Agilent Technologies India Pvt. Ltd, New Delhi, India).
- Microplate Reader (Molecular Devices, Sunnyvale, CA, USA)
- Gel scanner (Cannon Inc., Gurgaon, India).
Micropipettes- single channel: 20 µL, 100 µL, 200 µL, 1 mL & multi-channel-50 µL and 300 µL (Gilson, Inc Middleton, USA)

Steripette (Tarsons Product Pvt. Ltd., Kolkatta, India)

Repeat Pipette (Gilson, Inc Middleton, USA)

Reagent Reservoir, Utility tray (Tarsons Product Pvt. Ltd., Kolkatta, India)

Chromatography systems, Akta Explorer, Akta purifier & Akta Prime (GE Healthcare, Chennai, India)

Peristaltic pump (Cole Parmer India, Mumbai, India)

Glass column BPG 100 x 30 cm, Glass column XK 50 x 30 cm, Glass column XK 50 x 30 cm, Glass column XK 50 x 20 cm, Glass column XK 50 x 60 cm, Glass column XK 26/20 and Glass column XK 26 x 70 cm (GE Healthcare, Chennai, India)

Tubing and connectors for Chromatography systems (GE Healthcare, Chennai, India)

N-terminal Amino acid sequencer (Applied BioSystems, CA, USA)

Gel plates: plate size is 100 x 120 mm with 1 mm thick spacer, 6 well comb (maximum well capacity is 50 µL) or 8 well comb (maximum well capacity is 35 µL), Gel size, 90 x 90 mm with 1 mm thick spacer, Mini gel running unit, Submerge electro-transfer unit, Power pack (Bio-Rad Laboratories, Hercules, CA, USA)

UV-VIS Spectrophotometer (BioTek Instruments Inc., VT, USA)

Vortex mixer (Gilson, Inc., Middleton, WI, USA)