

## **CHILDREN'S HOSPITAL LOS ANGELES STEM CELL CORE ACCESS POLICY**

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## OVERVIEW

The Children's Hospital Los Angeles Stem Cell Core is a comprehensive support facility and central hub for the dissemination of resources, knowledge and state of the art equipment. Our goal is to accelerate research at Children's Hospital Los Angeles and the surrounding LA area by providing cells, reagents, training, assistance and laboratory space to investigators working with (pluripotent) stem cells. The Core offers the following facilities and services:

- Quality controlled cells and reagents
- State of the art analytical and cell culture equipment
- Communal laboratory space
- Training
- Technical assistance
- Educational documents for maintaining and working with pluripotent stem cells
- In addition to aforementioned services, the Children's Hospital Los Angeles Stem Cell Core facility is also involved in a variety of research activities:

The Core facility is managed by Laura-Marie Nucho and operated by a technical staff consisting of Laura-Marie Nucho and Sarah Webster. The activities of the core are funded in part by the California Institute For Regenerative Medicine (CIRM) and the Saban Research Institute at Children's Hospital Los Angeles. The remaining running costs are subsidized through nominal service fees for cells, reagents, equipment, training and technical assistance.

The Core facility is equipped with private laboratory space designated for use by only the Stem Cell Core staff and communal areas for researchers to conduct pilot and collaborative research. The shared laboratory space houses five cell culture work stations, tissue culture microscopes, an inverted fluorescence microscopy system, real time PCR equipment, and nucleofection technology. The Stem Cell Core also possesses flow cytometry equipment, which is managed by the Children's Hospital Los Angeles Flow Cytometry Core.

## ESTABLISHING AN ACCOUNT

New clients are required to establish an account by returning a completed copy of the Children's Hospital Los Angeles Stem Cell Core contact information form to the facility manager, Laura-Marie Nucho ([LNucho@chla.usc.edu](mailto:LNucho@chla.usc.edu)). This form can be downloaded from the Ordering Cells and Reagents webpage and must be completed prior to requesting services. We require all clients to update their contact and funding information on an annual basis.

## REQUESTING CELLS

To request cells from the Children's Hospital Los Angeles Stem Cell Core you must be registered as a client of the facility. Please refer to New clients portion of the Ordering Cells and Reagents web page for information on establishing an account.

Clients with an active account may order cells using the Children's Hospital Los Angeles Stem Cell Core website according to the posted instructions. Orders are submit via cell request forms, which are downloaded from the site and emailed to [stemcellcore@chla.usc.edu](mailto:stemcellcore@chla.usc.edu).



#### Please Note:

Orders to the Core are processed Monday through 10am Friday.  
Orders received after 10am Friday will be processed the following week.  
Conformation will be sent via email once your order has been processed  
Prior notice is required for all live cell orders (please see website for prior notice times)

#### Requesting human pluripotent stem cells

Researchers must obtain approval from the Children's Hospital Los Angeles Stem Cell Research Oversight (SCRO) committee and complete a material transfer agreement in order to request human pluripotent stem cells from the Children's Hospital Los Angeles Stem Cell Core. Please refer to New clients portion of the Ordering Cells and Reagents web page for further details.

#### REQUESTING REAGENTS

##### USC Bioreagents Cabinet and Roche Freezer program

The Children's Hospital Los Angeles Stem Cell Core houses the USC Bioreagents cabinet and Roche freezer programs are run by the USC Norris Cancer Center Bioreagents Core. Please visit their website for information on their operating policies and procedures.  
<http://uscnorriscancer.usc.edu/Core/Bioreagent/default.aspx>

##### Other reagents

To request reagents from the Children's Hospital Los Angeles Stem Cell Core you must be registered as a client of the facility. Please refer to the New clients portion of the Ordering Cells and Reagents web page for information on establishing an account.

Clients with an active account may order cells using the Children's Hospital Los Angeles Stem Cell Core website according to the posted instructions. Orders are submit via reagent request forms, which are downloaded from the site and emailed to [stemcellcore@chla.usc.edu](mailto:stemcellcore@chla.usc.edu). Reagents distributed directly by the Children's Hospital Los Angeles Stem Cell Core include:

- Complete media (serum free hESC media, serum containing hESC media and MEF-CM\*)
- Knock out serum replacer
- Serum
- Antibodies

\*Prior notice of 1 week is required for orders of MEF-Conditioned Medium (MEF-CM).

#### COLLECTION INSTRUCTIONS

##### Local Clients

Orders placed by clients located within the Los Angeles area must be collected in person or by a designated colleague.

##### Long distance clients

Shipping is available for long distance clients located outside of the Los Angeles area for some items (to be determined on a case by case basis).



Shipping and handling costs are payable by the client.

Item	Collection instructions	Action if not collected
Live cells	Must be collected on the 'due date' submitted at the time of ordering.	Uncollected cells will be kept for 2 days then discarded and charge for at the regular rate.
Frozen cells	May be collected once email conformation of the order is received.	None
MEF-CM	Must be collected on the 'due date' submitted at the time of ordering.	Uncollected media will be kept for 1 week then discarded and charge for at the regular rate.
Complete media	May be collected once email conformation of the order is received.	Uncollected media will be kept for 1 week then discarded and charged for at the regular rate.
Serum and KSR	May be collected once email conformation of the order is received.	None
Antibodies	May be collected once email conformation of the order is received.	None

#### COLLECTION TIMES

All orders must be collected during the following times:

MONDAY: 10AM-4PM  
TUESDAY: 10AM-4PM  
WEDNESDAY: 10AM-4PM  
THURSDAY: 10AM-4PM  
FRIDAY: 10PM-2PM

#### EQUIPMENT USAGE

To utilize equipment at the Children's Hospital Los Angeles Stem Cell Core you must be registered as a client of the facility. Please refer to New clients portion of the Ordering Cells and Reagents web page for information on establishing an account.

Clients with an active account may book equipment via the Children's Hospital Los Angeles Stem Cell Core website according to the posted instructions. Training is provided for complex equipment and is requirement for novice researchers. Equipment usage and operating instructions are posted next to all major pieces of equipment and MUST be complied with at all times. Clients of the Children's Hospital Los Angeles Stem Cell Core using the microscopy equipment must sign an Acceptance of Responsibility form, and abide by the microscopy policies. Clients are expected to ask for assistance if they are unable to operate the equipment and required to report broken equipment and negligent users. Failure to comply may result in a suspension of services.



## REQUESTING LABORATORY SPACE

Please contact the Core manager Laura-Marie Nucho ([LNucho@chla.usc.edu](mailto:LNucho@chla.usc.edu)) to request communal laboratory space.

## REQUESTING TECHNICAL ASSISTANCE

Please contact the Core manager Laura-Marie Nucho ([LNucho@chla.usc.edu](mailto:LNucho@chla.usc.edu)) to request technical assistance.

## BIOBANKING

To request biobanking assistance you must be registered as a client of the facility. Please refer to New clients portion of the Ordering Cells and Reagents web page for information on establishing an account. Clients with an active account may request biobanking services via the Children's Hospital Los Angeles Stem Cell Core website according to the posted instructions. We highly recommend all cell lines are tested for mycoplasma prior to cryopreservation. We will not preserve lines that are known to be mycoplasma positive. Clients are required to read disclaimers relating to the biobanking service, which are posted in the Biobanking section of the Research Services web page.

## TRAINING

### One-on-One training

The Children's Hospital Los Angeles Stem Cell Core provides one-on-one training in a range of human pluripotent stem cell techniques. This training is available to researchers and students from around the world. Participants must have prior cell culture experience as the maintenance of human pluripotent stem cells involves advanced techniques.

### Structured courses

Children's Hospital Los Angeles offers a 2.5 day workshop for researchers interested in human pluripotent stem cell (hPSC) techniques aimed at researchers who are interested in performing experiments with hPSC. This course is available to all researchers and students from around the world. The culture of human pluripotent stem cells involves advanced techniques and students attending the course must have basic cell culture experience. For more information, please go to the Technical Assistance and Training page on the Children's Hospital Los Angeles Stem Cell Core website, or contact the Children's Hospital Los Angeles Stem Cell Core manager Laura-Marie Nucho ([LNucho@chla.usc.edu](mailto:LNucho@chla.usc.edu)).

The USC Stem Cell Core at the USC Health Sciences campus offers a five-day course in human pluripotent stem cell culture techniques. This course is available to all researchers and students from around the world. The culture of human pluripotent stem cells involves advanced techniques. Students attending the course must have basic cell culture experience. For more information, please go to the USC Stem Cell Core website, <https://sites.google.com/site/uscstemcellcorefacility/>, or contact the USC Stem Cell Core director, Dr. Victoria Fox ([victorif@med.usc.edu](mailto:victorif@med.usc.edu)).

### Customized Training

The Children's Hospital Los Angeles Stem Cell Core offers one-on-one training in a range of human pluripotent stem cell techniques. Students must have basic cell culture experience. Contact the stem cell



## PROTOCOLS

The Children's Hospital Los Angeles Stem Cell Core distributes a variety of protocols and instructional booklets for maintaining human pluripotent stem cells, which are distributed freely via our Website. These protocols reflect the methodologies used by the Children's Hospital Los Angeles and USC Stem Cell Cores. The guidelines written within them are based on observation made at the USC Stem Cell Core Facility. We acknowledge that there are many relevant methodologies published by other facilities, in addition to the protocols we provide. We do not guarantee our methodologies will work outside of the facility.

## PUBLICATIONS INVOLVING THE CORE

Stem Cell Core staff who contribute to the acquisition and analysis of experimental data, beyond the provision of cells, reagents, training and paid technical assistance, must be listed as an author on any resulting publications including abstracts, posters, talks, journal articles and reviews. All journal articles involving the Core must be reviewed by the manager prior to submission.

Individuals requiring information on cells, quality control analysis or equipment used in experimental research resulting in publication should contact the Stem Cell Core manager.

## ACKNOWLEDGING THE CORE

Clients receiving services from the Children's Hospital Los Angeles Stem Cell Core are required to acknowledge the facility according to the posted instructions on the Acknowledging the Core web page. The following text is provided for use in publications:

**This work was supported by The Children's Hospital Los Angeles Stem Cell Core Facility. The Children's Hospital Los Angeles Stem Cell Core is a CIRM funded shared laboratory.**

Please inform the Core manager when you have been published: LNucho@chla.usc.edu.

## REGULATIONS FOR WORKING AT THE Children's Hospital Los Angeles STEM CELL CORE LABORATORY

All researchers working within the Children's Hospital Los Angeles Stem Cell Core communal laboratory space must read and abide by the following regulations.

Safety training requirements: Children's Hospital Los Angeles investigators

Children's Hospital Los Angeles investigators are required to take laboratory safety training and receive permission from their PI in order to work at the Children's Hospital Los Angeles Stem Cell Core. Researchers working with live cells must undergo blood borne pathogen training. Please visit the Children's Hospital Los Angeles CHEX system, enroll and successfully complete the Bloodborne pathogen training module.

Safety training requirements: Non- Children's Hospital Los Angeles investigators

The Children's Hospital Los Angeles Stem Cell Core is a shared laboratory, and is available for use by Non- Children's Hospital Los Angeles researchers. All visitors and volunteers are required to sign in with the receptionist at the Saban Research Institute building or at the main hospital and acquire a visitor pass before working in the Stem Cell Core. Non-Children's Hospital Los Angeles visitors may be required to undergo safety training via the



Children's Hospital Los Angeles Stem Cell Core staff or the department of Environmental Health and Safety, depending on their duration at the facility (to be determined on a case by case basis).

#### General laboratory regulations

1. Comply with all posted guidelines for working safely at the Children's Hospital Los Angeles Stem Cell Core
2. Do not eat or drink in the laboratory or at the desk areas
3. Wash your hands thoroughly using antibacterial soap before entering and leaving the laboratory
4. Personal protective equipment must be worn when appropriate
5. Keep a clean and tidy working environment at all times
6. Do not run in the lab
7. Do not block access and exit routes
8. Ensure you know where the Emergency exit, fire extinguishers, chemical spill kit, eye wash and medical kits are located
9. Consult with a member of the Children's Hospital Los Angeles Stem Cell Core staff if you in doubt of safe working practices
10. Work considerately

#### Personal protective equipment

The following personal protective equipment must be worn as stated in the table below:

Lab Coat	At all times
Gloves	At all times
Face mask	Clean bench
Close toe shoes	At all times
Face shield	When working with Liquid Nitrogen
Cryogloves	When working with Liquid Nitrogen

#### Working safely with Sharps

Sharp objects include needles, glass pipettes, scalpels, tweezers and scissors

1. Use engineered safety sharps whenever possible.
2. Take extra care when handling sharp objects.
3. Do not re-sheath needles or any other sharp object.
4. Dispose of waste sharps in the sharps bin provided.
5. Do not insert your hand into the sharps bin for any reason.
6. Ensure the lid to the sharps bin is closed when not in use.
7. Do not rest equipment or other items on the sharps bin.
8. Do not remove needles from the syringe barrel prior to disposal.
9. When cutting cells, insert the needle into the center well of the dish while observing the dish. Do not place the needle into the well while looking into the microscope objective.
10. Wash your hands thoroughly 10-15 times with antibacterial soap, then consult with the Children's Hospital Los Angeles Stem Cell Core staff immediately if you are injured by a sharp object.



## PROCEDURES FOR WORKING WITH LIVE CELLS

### General guidelines

1. All researchers working with live cells must have experience culturing cells and working aseptically. Novice researchers may be required to undergo training by the Stem Cell Core staff if they do not have prior experience.
2. Children's Hospital Los Angeles Investigators are required to have up to date blood borne pathogen training in order to work with live cells. Please visit the Children's Hospital Los Angeles CCI webpage, click to the [HSPP Certification and Education Courses](#) and register with CITI (<http://www.citiprogram.org/>) if this has not been done already. Affiliate with "University of Southern California-Laboratory Practices" (NOT "University of Southern California"). Select "OSHA Bloodborne Pathogens" training module and complete the course and quiz.
3. Investigators are required to comply with the Children's Hospital Los Angeles Stem Cell Core's Cell handling procedures.
4. Investigators are required to follow the Children's Hospital Los Angeles Stem Cell Core's guidelines for working with biosafety cabinets and clean benches.
5. Investigators are required to comply with the Children's Hospital Los Angeles Stem Cell Core's guidelines for preventing and eliminating contaminations.
6. Individuals who do not comply with the Stem Cell Cores operating procedures for working with live cells will not be permitted to work at the Core facility.

### Blood borne pathogen exposure program

In working with animal cells you are exposed to the pathogens those cells may harbor. To minimize the risk of pathogen exposure it is imperative to observe the Children's Hospital Los Angeles bloodborne pathogen exposure program as outlined below:

1. Undergo annual bloodborn pathogen training through CITI (Children's Hospital Los Angeles investigators only).
  2. Ensure you have read and understood the Children's Hospital Los Angeles Bloodborne pathogen program exposure control plan.
  3. If you are a Children's Hospital Los Angeles staff or student, ensure you have received the option of Hep B vaccination.
  4. Adhere to ALL of the safety and equipment usage guidelines posted around the laboratory.
  5. Comply with the Children's Hospital Los Angeles Stem Cell Core cell handling procedures and biosafety cabinet operating instructions at all times. These are designed to minimize the risk of exposure to blood borne pathogens.
  6. Conduct all work involving tissues and cell cultures, human or otherwise in the biosafety cabinets.
  7. Work only with cells authorized for use by the Core Facility.
  8. Dispose of biohazardous waste according to the posted guidelines.
  9. Wear the correct PPE and conduct frequent hand washing.
  10. Maintain a clean working environment, clean any spills immediately.
  11. Ensure all biohazardous spills are cleaned thoroughly with 70% alcohol or Bleach.
  12. Do not work with primary tissues (human or otherwise), without first consulting with the Children's Hospital Los Angeles Stem Cell Core staff and undergoing bloodborne pathogen training.
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13. If you are exposed to biohazardous material, wash the affected area 10-15 times with antibacterial soap and report immediately to a member of the Children's Hospital Los Angeles Stem Cell Core.
14. Consult with a member of the Children's Hospital Los Angeles Stem Cell Core staff if you are concerned or require further advice regarding bloodborne pathogen exposure.

#### Use of antibiotics

We do not recommend the use of antibiotics when maintaining immortalized cell lines undergoing long term culture for the following reasons:

- 1) They do not guard against all bacteria and fungi,
- 2) They can slow the growth of some microbes without completely eliminating them,
- 3) They can mask the presence of some microbial contaminants. Cultures maintained with antibiotics can contain microbial contaminations unbeknown to the investigator.

This results in the prolonged culture and cryopreservation of contaminated cell lines. We advocate the use of antibiotics only for protecting experiments and primary cell lines with a short and finite life span, if required.

#### Authorized cell lines

All cell culture must be carried out using quality-controlled cells distributed by the Children's Hospital Los Angeles Stem Cell Core, unless permitted by the Children's Hospital Los Angeles Stem Cell Core manager. Authorized cell lines include live and frozen cells available via the Children's Hospital Los Angeles Stem Cell Core website.

#### Outside cell lines

Investigators may bring cell lines into the Children's Hospital Los Angeles Stem Cell Core following approval from the Core manager. Outside lines are required to undergo mycoplasma testing and be worked with in the quarantine area until the testing is complete. It is the responsibility of the researcher to cover the cost of mycoplasma testing. The Children's Hospital Los Angeles Stem Cell Core is a mycoplasma free zone. Cell lines that are found to harbor mycoplasma must be removed from the facility immediately.

#### Deriving cell lines at the Children's Hospital Los Angeles Stem Cell Core

Researchers must obtain approval from the Children's Hospital Los Angeles Stem Cell Core manager, before deriving new cell lines at the facility. Derivation and routine culture of the resulting lines must be carried out in the quarantine area in a class II biosafety cabinet. Researches are required to mycoplasma test newly derived cell lines. We do not permit mycoplasma positive cultures to be maintained at the Children's Hospital Los Angeles Stem Cell Core.

#### WORKING WITH TISSUE

Researchers must obtain approval from the IRB and the Children's Hospital Los Angeles Stem Cell Core manager before working with human or animal tissue at the facility. Work involving tissues must be carried out in the quarantine area in a class II biosafety cabinet.



## WORKING WITH ANIMALS

Researchers must obtain approval from the IACUC and the Children's Hospital Los Angeles Stem Cell Core manager before working with live animals at the facility.

## WORKING WITH VIRUSES

1. Work involving replication deficient lentivirus is permitted at the Children's Hospital Los Angeles Stem Cell Core and must be carried out in the quarantine area.
2. Work involving viruses other than lentivirus must be approved by the Children's Hospital Los Angeles Stem Cell Core manager.
3. Researchers must have approval from the IBC to work with viruses at the Children's Hospital Los Angeles Stem Cell Core.
4. Researchers must have experience conducting viral work.
5. All viral work must be carried out with biohazard level II replication deficient viruses in the quarantine area in a class II biosafety cabinet.

## WORKING WITH HAZARDOUS CHEMICALS

The following hazardous chemicals are approved for use at the Children's Hospital Los Angeles Stem Cell Core as working solutions. **As there are no fume hoods** in the Children's Hospital Los Angeles stem cell core, researchers are not permitted to make working solutions in the Core facility. Researchers must obtain permission from the Core director before working with unauthorized hazardous material.

### Please note:

- **Ethidium bromide is not permitted** in the Stem Cell Core Facility. Please use an alternate gel dye, such as SYBR Safe DNA gel from Life Technologies, or GelRed from Biotium.
- Paraformaldehyde (PFA) powder is not allowed in the Stem Cell Core facility. Only working PFA solutions is allowed for use in the Core facility.

Mitomycin C	Paraformaldehyde Solution	Ammonium persulphate
Acetone	Acrylamide	

## WASTE DISPOSAL PROCEDURES

Waste materials must be disposed of according to the instructions in the table below.

### Chemicals

Please consult with a member of the Children's Hospital Los Angeles Stem Cell Core for chemical disposal instructions

Biohazards (Agents which may cause disease in humans, significant environmental impact, human or primate tissues, cells and any agents that have been in contact with cells, in some cases animal derived products)

Gloves	Large Red plastic biohazard bag
Disposable lab coats	Large Red plastic biohazard bag
Cell culture media, sera, KSR and any media components containing animal or human products.	Inactivate with bleach for 30 minutes then dispose of via sink. Cell culture medium taken directly from cells should be disposed of via the vacuum line into the glass bottle located

	under the Biosafety cabinet
Serological pipettes	Large Red plastic biohazard bag
Filter units	Large Red plastic biohazard bag
Tubes	Large Red plastic biohazard bag
Flasks and dishes of cells	Remove cell culture medium into bleach, inactivate in bleach for 30 minutes then discard into red plastic biohazard bag
Cell scrapers	Large Red plastic biohazard bag
Pipettor tips	Large Red plastic biohazard bag
Recognizable human tissue	Place in a leak proof container provided EH&S and call for disposal (323 361 2943)
Non infected non human animal tissue (unfixed)	Brown paper bag obtained from vivarium. Take to vivarium for disposal.
Non infected non human animal tissue (fixed)	Large Red plastic biohazard bag

#### Sharps (biohazardous and non biohazardous)

Needles, glass pipettes, tweezers, scissors, scalpels	Red sharps bin
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#### Non hazardous waste

Empty PBS and basal media bottles	Trash bin
Xeno free non hazardous media components (i.e. basal media)	Sink
Plastic packaging	Trash or recycle bin

#### Non hazardous glass bottles

Card glass disposal bin
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(Please consult with Children's Hospital Los Angeles Stem Cell Core Staff if the bottle previously contained a harmful chemical or if you are unsure of how it should be disposed)

## EMERGENCY PROCEDURES

### Fire

If the event of a fire or fire alarm, exit the building quickly and calmly by the stairs. Do not stop to pick up personal belonging or take the elevator. Gather in the VONS parking lot at a safe distance from the building.

### Earthquake

In the event of a major earthquake, take cover under something solid like a door frame or table until the shaking stops then exit the building via the stairs and gather in the VONS parking lot at a safe distance. If a minor earthquake occurs exit the building calmly via the stairs and gather in the VONS parking lot. Elevators will not be operational and should not be used during an earthquake.

### Injury

If a serious injury occurs (Children's Hospital Los Angeles and non- Children's Hospital Los Angeles personnel) call 33 or Security at 711 or extension 1-2313.

#### Sharps injury:

1. Remove PPE
2. Wash puncture site for a minimum of 15-20 minutes with antibacterial soap and water
3. Report the injury immediately to a member of the Stem Cell Core Staff
4. Report for medical attention if the sharp was exposed to a hazardous substance (biohazard or chemical)

#### Exposure to eyes, nose, mouth or other mucous membranes

1. Remove PPE
2. Wash site for a minimum of 15-20 minutes at an eye wash station
3. Report the injury immediately to a member of the Stem Cell Core Staff
4. Report for medical attention if exposed to a hazardous substance (biohazard or chemical)

#### Exposure to body or extremities

1. Remove PPE/contaminated clothing
2. Wash site for a minimum of 15-20 minutes with antibacterial soap and water, at a shower if necessary
3. Report the injury immediately to a member of the Stem Cell Core Staff
4. Report for medical attention hazardous substance (biohazard or chemical)

#### Medical attention

1. Children's Hospital Los Angeles staff: Refer to Children's Hospital Los Angeles Employee Health Department, 4601 Sunset Blvd, corner of Sunset Blvd, and Rodney. 323.361.2533
2. Children's Hospital Los Angeles student: Refer to Children's Hospital Los Angeles Employee Health Department, 4601 Sunset Blvd, corner of Sunset Blvd, and Rodney. 323.361.2533
3. Non-Children's Hospital Los Angeles personnel: Refer to personal physician for advice.

#### SERVICE FEES

The Stem Cell Core is funded partly by the California Institute for Regenerative medicine (CIRM) and partly by the Saban Research Institute at Children's Hospital Los Angeles. The remaining running costs are subsidized through chargeback for cells, supplies, equipment, technical assistance, consultation and training. All clients receiving these services are required to pay service fees or provide sufficient funding to cover the cost of salaries, supplies and equipment maintenance, following consultation and approval from the Core manager Laura-Marie Nucho ([LNucho@chla.usc.edu](mailto:LNucho@chla.usc.edu)). Researchers requiring extensive or ongoing services may consult with the Core manager to negotiate a bulk package. Failure to pay may result in suspended services. Please refer to Service fees for further information on fee structure.

#### BILLING AND PAYMENT

Clients of the Children's Hospital Los Angeles Stem Cell Core are billed monthly and must submit payment within 28 days of receiving an invoice. Failure to pay may result in suspended services. Please refer to Billing & Payment for further information on billing procedures.

