# $^{68}$Ga-Glutamate peptide-3 aminoethyl estradiol

## $^{68}$Ga-GAP-EDL

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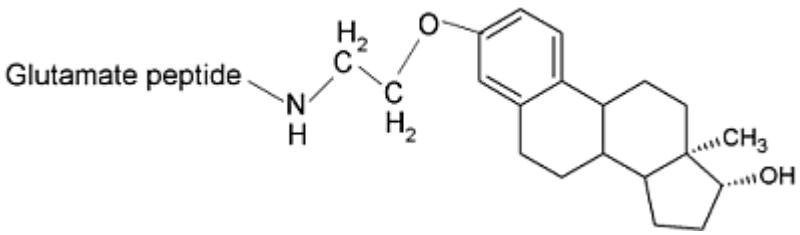
| **Chemical name:** | $^{68}$Ga-Glutamate peptide-3 aminoethyl estradiol |
| **Abbreviated name:** | $^{68}$Ga-GAP-EDL |
| **Synonym:** | |
| **Backbone:** | Estradiol |
| **Target:** | Estrogen receptor |
| **Mechanism:** | Receptor-ligand binding |
| **Method of detection:** | Positron emission tomography (PET) |
| **Source of signal:** | $^{68}$Ga |
| **Activation:** | Not required |
| **Studies:** | • *In vitro*<br>• Rodents |




Structure of GAP-EDL. The exact location of $^{68}$Ga in the structure is unknown.

# Background

[PubMed]

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Approximately 10% of women of reproductive age are affected by endometriosis, a gynecological disorder (1). Painful periods, pelvic pain, and infertility are often associated with endometriosis. Although a variety of mechanisms, including the roles of different cytokines, growth factors, and hormones, have been proposed in the development of endometriosis, the exact etiology and epidemiology of this condition is unclear (1-3). Menstruation in the opposite direction with spillage of viable endometrial cells into the peritoneum is often observed in cycling women (4). Adhesion, proliferation, and spread of the shed endometrial cells to the peritoneum are believed to support the development of endometriosis (5-7). Endometriosis is usually detected by a surgical technique such as a laparoscopy. Performing such a procedure on patients with infertility is often complex, and it provides information only about the morphological and anatomical changes in the tissue (8). Moreover, endometriosis usually progresses or recurs after surgery, and any relief to the patient is temporary (9).

Because the prevalence and progression of endometriosis cannot be studied in humans without a laparoscopy, non-primate and primate animal models, including rabbits, have been used to investigate this disease (10-12). It has also been shown that estrogen receptors (ER) are overexpressed in the uterine endometrium and endometriotic lesions in endometriosis (4). In an effort to develop a non-invasive method to detect endometriosis, Takahashi et al. explored the use of estrogen, an ER ligand, to image endometriosis induced in rabbits (8). For this, estradiol (EDL) was conjugated to a glutamate peptide (GAP), which contained 5–10 glutamic acid residues, to facilitate the chelation of radioactive gallium ($^{68}$Ga) to obtain $^{68}$Ga-GAP-EDL to image endometriosis.

## Synthesis

[PubMed]

The synthesis of $^{68}$Ga-GAP-EDL was performed as described by Takahashi et al. (8, 13). To start, estrone was dissolved in anhydrous ethanol. Sodium ethoxide and bromoacetonitrile were added to the solution and the mixture was heated under reflux for 3 h. The ethanol was allowed to evaporate completely and the residue was dissolved in ethyl acetate. The solution was washed with water and the organic layer was filtered after drying over magnesium sulphate. The ethyl actetate was evaporated under reduced pressure and the remaining solid product, 3-acetonitrile estradiol, was washed with ether. The yield of this reaction was 75%. The solid product was dissolved in tetrahydrofuran, and lithium aluminum hydride was added to the mixture and stirred for 4 h. The solvent was evaporated to yield a solid that was dissolved in ethyl acetate. This solution was washed with water, dried over magnesium sulphate, and filtered. The solvent was evaporated to obtain 3-cyanomethyl estrone (EDL) with a yield of 92%. The structure of EDL was confirmed by nuclear magnetic resonance.

For the synthesis of GAP-EDL, the sodium salt of GAP was converted to the acid form by the addition of 2 N hydrochloric acid (HCl). This solution was dialyzed to remove all the acid and freeze-dried to obtain the acidic form of GAP. The GAP acid was dissolved in

dimethylformamide (DMF), and dicyclohexyl carbodiimide and 4-*N*,*N*-dimethyl aminopyridine were added to it. This mixture was stirred at room temperature for 2 days. The DMF was evaporated under reduced pressure; 1 N sodium bicarbonate was added to it and extracted with chloroform. The aqueous solution was dialyzed, filtered, and freeze-dried to obtain GAP-EDL. The percent yield of this reaction was not provided in the publication. The GAP-EDL complex contained 7% (weight/weight) EDL as determined by ultraviolet spectroscopy.

For the synthesis of $^{68}$Ga-GAP-EDL, $^{68}$Ga was eluted from a $^{68}$Ge/$^{68}$Ga generator with 0.1 N HCl, and the acidic solution was passed through an anion resin cartridge to trap the $^{68}$Ga. The cartridge was washed with 4 N HCl and air-dried. The $^{68}$Ga was eluted as $^{68}$Ga-chloride ($^{68}GaCl_3$) from the cartridge with water, and the pH was adjusted to 4–5 with sodium hydroxide and sodium acetate. GAP-EDL in acetate buffer was added to the $^{68}GaCl_3$ solution, and the reaction was completed by warming at 37°C for 20 min. Radiochemical purity of the product was determined by instant thin-layer chromatography. The Rf values for GAP-EDL and $^{68}GaCl_3$ were reported to be 0.1 and 0.9, respectively. The radiochemical purity of the radiotracer was >95%. Specific activity of the radiotracer was not provided in the publication (8).

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

The uptake of $^{68}$Ga-GAP-EDL was studied in the 13762 rat adenocarcinoma cell line (8), and accumulation of the radiopharmaceutical in these cells was shown to increase with time. Addition of cold estrone (estradiol) inhibited the uptake in a dose-dependent manner. This indicated that the uptake of $^{68}$Ga-GAP-EDL in the cells was an ER-mediated process.

## Animal Studies

### Rodents

[PubMed]

No publications are currently available.

### Other Non-Primate Mammals

[PubMed]

The uptake of $^{68}$Ga-GAP-EDL was investigated in rabbits with induced endometriosis (8). Endometrial tissues were implanted in the rabbits, and the implanted endometrial tissue was microscopically visible in the animals after 8 weeks. The uptake of $^{68}$Ga-GAP-EDL by the rabbits was investigated with and without the treatment of tamoxifen, a competitive inhibitor of estrogen for binding to the ER. A cross-sectional review of the coronal and sagittal images taken by positron emission tomography showed that the endometrial

implant tissue could be differentiated from the skeletal, hepatobiliary, and urinary uptake of the label. Prior treatment of the animals with tamoxifen resulted in the inhibition of label uptake by the endometrial tissue. This demonstrated that the uptake of $^{68}$Ga-GAP-EDL in the endometrial implants was ER-mediated. From this observation, the investigators suggested that $^{68}$Ga-GAP-EDL can be used as a non-invasive imaging agent for the detection and monitoring of endometriosis and other diseases associated with the overexpression of ER, such as breast cancer .

### Non-Human Primates

[PubMed]

No publications are currently available.

## Human Studies

[PubMed]

No publications are currently available.

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