UNITED STATES SECURITIES AND EXCHANGE COMMISSION

Washington, D.C. 20549

Amendment No. 1 to

FORM S-3

REGISTRATION STATEMENT UNDER THE SECURITIES ACT OF 1933

LIXTE BIOTECHNOLOGY HOLDINGS, INC.

(Exact name of registrant as specified in its charter)

Delaware (State or other jurisdiction of incorporation or organization)

X

(Check one):

Large accelerated filer

Non-accelerated filer

20-2903526 (I.R.S. Employer Identification No.)

Accelerated filer

Smaller reporting company

Emerging growth company

X

680 East Colorado Boulevard, Suite 180
Pasadena, California 91101
(631) 830-7092
(Address, including zip code, and telephone number, including area code, of registrant's principal executive offices)

Bas van der Baan
Chief Executive Officer
Lixte Biotechnology Holdings, Inc.
680 East Colorado Boulevard, Suite 180
Pasadena, California 91101
(631) 830-7092
(Name, address, including zip code, and telephone number, including area code, of agent for service)

With copies to:

David Ficksman TroyGould PC 1801 Century Park East 16th Floor Los Angeles, California 90067 (310) 553-4441

Approximate date of commencement of proposed sale to the public:

Approximate date of commencement of proposed safe to the public.
From time to time after the effective date of this registration statement.
If the only securities being registered on this Form are being offered pursuant to dividend or interest reinvestment plans, please check the following box. \Box
If any of the securities being registered on this Form are to be offered on a delayed or continuous basis pursuant to Rule 415 under the Securities Act of 1933, other than securities offered only in connection with dividend or interest reinvestment plans, check the following box.
If this Form is filed to register additional securities for an offering pursuant to Rule 462(b) under the Securities Act, please check the following box and list the Securities Act registration statement number of the earlier effective registration statement for the same offering. \Box
If this Form is a post-effective amendment filed pursuant to Rule 462(c) under the Securities Act, check the following box and list the Securities Act registration statement number of the earlier effective registration statement for the same offering. \Box
If this Form is a registration statement pursuant to General Instruction I.D. or a post-effective amendment thereto that shall become effective upon filing with the Commission pursuant to Rule $462(e)$ under the Securities Act, check the following box. \Box
If this Form is a post-effective amendment to a registration statement filed pursuant to General Instruction I.D. filed to register additional securities or additional classes of securities pursuant to Rule 413(b) under the Securities Act, check the following box. \Box
Indicate by check mark whether the registrant is a large accelerated filer, an accelerated filer, a non-accelerated filer, a smaller reporting company, or an emerging growth

company. See the definitions of "large accelerated filer", "accelerated filer", "smaller reporting company" and "emerging growth company" in Rule 12b-2 of the Exchange Act.

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 7(a)(2)(B) of the Securities Act. \Box

The registrant hereby amends this registration statement on such date or dates as may be necessary to delay its effective date until the registrant shall file a further amendment which specifically states that this registration statement shall thereafter become effective in accordance with Section 8(a) of the Securities Act of 1933 or until the registration statement shall become effective on such date as the Commission, acting pursuant to said Section 8(a), may determine.

EXPLANATORY NOTE

The sole purpose of this Amendment No. 1 to the Registration Statement on Form S-3 of Lixte Biotechnology Holdings, Inc. (the "Company") is to file a revised Exhibit 5.1. Accordingly, this Amendment No. 1 consists only of the facing page, this explanatory note, Part II, including the signature page and the Exhibit Index, and the revised Exhibit 5.1 filed herewith. This Amendment No. 1 does not contain the prospectuses that were included in the Registration Statement and is not intended to amend or delete any part of any of the prospectuses.

PART II

INFORMATION NOT REQUIRED IN PROSPECTUS

Item 14. Other Expenses of Issuance and Distribution.

The following table sets forth the fees and expenses to be paid by us, other than underwriting discounts and commissions, in connection with the offering of the securities described in this registration statement. All amounts shown are estimates except for the SEC registration fee.

SEC registration fee	\$ 7,675.00
FINRA filing fee	*
Nasdaq listing fee	*
Printing expenses	*
Legal fees and expenses	*
Accounting fees and expenses	*
Transfer agent and registrar fees and expenses	*
Trustee fees and expenses	*
Depositary fees and expenses	*
Warrant agent fees and expenses	*
Miscellaneous expenses	*
Total	\$ *

^{*} These fees and expenses will be based upon the number of securities offerings and the amount of securities offered and accordingly cannot be estimated at this time.

Item 15. Indemnification of Directors and Officers.

Section 102 of the Delaware General Corporation Law (the "DGCL") permits a corporation to eliminate the personal liability of directors of a corporation to the corporation or its shareholders for monetary damages for a breach of fiduciary duty as a director, except where the director breached his or her duty of loyalty, failed to act in good faith, engaged in intentional misconduct or knowingly violated a law, authorized the payment of a dividend or approved a stock repurchase in violation of Delaware corporate law or obtained an improper personal benefit.

Section 145 of the DGCL provides that a corporation has the power to indemnify a director, officer, employee, or agent of the corporation, or a person serving at the request of the corporation for another corporation, partnership, joint venture, trust or other enterprise in related capacities, against expenses (including attorneys' fees), judgments, fines and amounts paid in settlement actually and reasonably incurred by the person in connection with an action, suit or proceeding to which he or she was or is a party or is threatened to be made a party to any threatened, ending or completed action, suit or proceeding by reason of such position, if such person acted in good faith and in a manner he or she reasonably believed to be in or not opposed to the best interests of the corporation, and, in any criminal action or proceeding, had no reasonable cause to believe his or her conduct was unlawful, except that, in the case of actions brought by or in the right of the corporation, no indemnification will be made with respect to any claim, issue or matter as to which such person has been adjudged to be liable to the corporation unless and only to the extent that the Delaware Court of Chancery or other adjudicating court determines that, despite the adjudication of liability but in view of all of the circumstances of the case, such person is fairly and reasonably entitled to indemnity for such expenses which the Delaware Court of Chancery or such other court deems proper.

II-1

Our Amended and Restated Bylaws allow for, and our Certificate of Incorporation, as amended, provides for, indemnification of our current and former directors, officers, committee members or representatives to the full extent permitted by the DGCL. In addition, our Certificate of Incorporation as amended, provides that to the fullest extent permitted by the DGCL, a director of Lixte Biotechnology Holdings, Inc. shall not be personally liable to us or our stockholders for monetary damages for breach of fiduciary duty as a director. These indemnification provisions may be sufficiently broad to permit indemnification of our officers and directors for liabilities (including reimbursement of expenses incurred) arising under the Securities Act. Our directors and officers are also covered by insurance policies maintained by us against certain liabilities for actions taken in their capacities as such, including liabilities under the Securities Act. The foregoing summary is qualified in its entirety by reference to the terms and provisions of such documents or arrangements referred above, including our Certificate of Incorporation, as amended, and our Amended and Restated Bylaws, to which you should refer and copies of which are incorporated herein by reference as Exhibits 3.1, 3.2 and 3.5, respectively, to the registration statement on Form S-3, of which this prospectus forms a part.

Item 16. Exhibits.

A list of the exhibits filed with this registration statement on Form S-3 is set forth on the Exhibit Index and is incorporated herein by reference.

- (a) The undersigned registrant hereby undertakes:
 - (1) To file, during any period in which offers or sales are being made, a post-effective amendment to this registration statement:
 - (i) To include any prospectus required by Section 10(a)(3) of the Securities Act of 1933;
- (ii) To reflect in the prospectus any facts or events arising after the effective date of the registration statement (or the most recent post-effective amendment thereof) which, individually or in the aggregate, represent a fundamental change in the information set forth in the registration statement; notwithstanding the foregoing, any increase or decrease in volume of securities offered (if the total dollar value of securities offered would not exceed that which was registered) and any deviation from the low or high end of the estimated maximum offering range may be reflected in the form of prospectus filed with the Commission pursuant to Rule 424(b) if, in the aggregate, the changes in volume and price represent no more than a 20 percent change in the maximum aggregate offering price set forth in the "Calculation of Registration Fee" table in the effective registration statement; and
- (iii) To include any material information with respect to the plan of distribution not previously disclosed in the registration statement or any material change to such information in the registration statement;

provided, however, that paragraphs (a)(1)(i), (a)(1)(ii) and (a)(1)(iii) above do not apply if the information required to be included in a post-effective amendment by those paragraphs is contained in reports filed with or furnished to the Commission by the registrant pursuant to Section 13 or Section 15(d) of the Securities Exchange Act of 1934 that are incorporated by reference in the registration statement, or is contained in a form of prospectus filed pursuant to Rule 424(b) that is part of the registration statement.

- (2) That, for the purpose of determining any liability under the Securities Act of 1933, each such post-effective amendment shall be deemed to be a new registration statement relating to the securities offered therein, and the offering of such securities at that time shall be deemed to be the initial bona fide offering thereof.
- (3) To remove from registration by means of a post-effective amendment any of the securities being registered which remain unsold at the termination of the offering.

11-2

- (4) That, for the purpose of determining liability under the Securities Act of 1933 to any purchaser:
- (A) Each prospectus filed by the registrant pursuant to Rule 424(b)(3) shall be deemed to be part of the registration statement as of the date the filed prospectus was deemed part of and included in the registration statement; and
- (B) Each prospectus required to be filed pursuant to Rule 424(b)(2), (b)(5), or (b)(7) as part of a registration statement in reliance on Rule 430B relating to an offering made pursuant to Rule 415(a)(1)(i), (vii), or (x) for the purpose of providing the information required by Section 10(a) of the Securities Act of 1933 shall be deemed to be part of and included in the registration statement as of the earlier of the date such form of prospectus is first used after effectiveness or the date of the first contract of sale of securities in the offering described in the prospectus. As provided in Rule 430B, for liability purposes of the issuer and any person that is at that date an underwriter, such date shall be deemed to be a new effective date of the registration statement relating to the securities in the registration statement to which that prospectus relates, and the offering of such securities at that time shall be deemed to be the initial bona fide offering thereof; *provided*, *however*, that no statement made in a registration statement or prospectus that is part of the registration statement or made in a document incorporated or deemed incorporated by reference into the registration statement or prospectus that is part of the registration statement will, as to a purchaser with a time of contract of sale prior to such effective date, supersede or modify any statement that was made in the registration statement or prospectus that was part of the registration statement or made in any such document immediately prior to such effective date.
- (5) That, for the purpose of determining liability of the registrant under the Securities Act of 1933 to any purchaser in the initial distribution of securities, the undersigned registrant undertakes that in a primary offering of securities of the undersigned registrant pursuant to this registration statement, regardless of the underwriting method used to sell the securities to the purchaser, if the securities are offered or sold to such purchaser by means of any of the following communications, the undersigned registrant will be a seller to the purchaser and will be considered to offer or sell such securities to such purchaser:
 - (i) Any preliminary prospectus or prospectus of the undersigned registrant relating to the offering required to be filed pursuant to Rule 424;
- (ii) Any free writing prospectus relating to the offering prepared by or on behalf of the undersigned registrant or used or referred to by the undersigned registrant;
- (iii) The portion of any other free writing prospectus relating to the offering containing material information about the undersigned registrant or its securities provided by or on behalf of the undersigned registrant; and
 - (iv) Any other communications that is an offer in the offering made by the undersigned registrant to the purchaser.
- (b) The undersigned registrant hereby undertakes that, for purposes of determining any liability under the Securities Act of 1933, each filing of the registrant's annual report pursuant to Section 13(a) or Section 15(d) of the Securities Exchange Act of 1934 (and, where applicable, each filing of an employee benefit plan's annual report pursuant to section 15(d) of the Securities Exchange Act of 1934) that is incorporated by reference in the registration statement shall be deemed to be a new registration statement relating to the securities offered therein, and the offering of such securities at that time shall be deemed to be the initial bona fide offering thereof.
- (c) Insofar as indemnification for liabilities arising under the Securities Act of 1933 may be permitted to directors, officers and controlling persons of the registrant pursuant to the foregoing provisions, or otherwise, the registrant has been advised that in the opinion of the Securities and Exchange Commission such indemnification is against public policy as expressed in the Securities Act of 1933 and is, therefore, unenforceable. In the event that a claim for indemnification against such liabilities (other than the payment by the registrant of expenses incurred or paid by a director, officer or controlling person of the registrant in the successful defense of any action, suit or proceeding) is asserted by such director, officer or controlling person in connection with the securities being registered, the registrant will, unless in the opinion of its counsel the matter has been settled by controlling precedent, submit to a court of appropriate jurisdiction the question whether such indemnification by it is against public policy as expressed in the Securities Act of 1933 and will be governed by the final adjudication of such issue.
- (d) The undersigned registrant hereby undertakes to file an application for the purpose of determining the eligibility of the trustee to act under subsection (a) of Section 310 of the Trust Indenture Act (the "Act") in accordance with the rules and regulations prescribed by the Securities and Exchange Commission under Section 305(b)(2) of the Act.

11-3

SIGNATURES

<u>LIXTE BIOTECHNOLOGY HOLDINGS, INC.</u> (Registrant)

By: /s/ Bas van der Baan

Bas van der Baan

President and Chief Executive Officer, and Chairman of the Board of Directors

POWER OF ATTORNEY

KNOW ALL BY THESE PRESENTS, that each person whose signature appears below constitutes and appoints Bas van der Baan as his or her true and lawful attorney-in-fact and agent, with the full power of substitution and resubstitution, for him or her and in his or her name, place or stead, in any and all capacities, to sign any and all amendments to this Registration Statement (including post-effective amendments), and to file the same with all exhibits thereto and other documents in connection therewith, with the Securities and Exchange Commission, granting unto said attorney-in-fact and agent, full power and authority to do and perform each and every act and thing requisite and necessary to be done in connection therewith, as fully to all intents and purposes as he or she might or could do in person, hereby ratifying and confirming all that said attorney-in-fact and agent, or his or her substitute or substitutes, may lawfully do or cause to be done by virtue hereof.

Pursuant to the requirements of the Securities Act of 1933, as amended, this Registration Statement on Form S-3 has been signed by the following persons in the capacities and on the dates indicated below.

Signature	Title	Date
/s/ BAS VAN DER BAAN Bas van der Baan	President and Chief Executive Officer, and Chairman of the Board of Directors (Principal Executive Officer)	April 30, 2024
/s/ ROBERT N. WEINGARTEN* Robert N. Weingarten	Vice President and Chief Financial Officer (Principal Financial and Accounting Officer)	April 30, 2024
/s/ RENE BERNARDS* Rene Bernards	Director	April 30, 2024
/s/ STEPHEN J. FORMAN* Stephen J. Forman	Director	April 30, 2024
/s/ REGINA BROWN* Regina Brown	Director	April 30, 2024
/s/ YUN YEN* Yun Yen	Director	April 30, 2024
*By Bas van der Baan, Attorney-in-Fact		
	II-4	

EXHIBIT INDEX

Exhibit Number	Description of Document
	· ·
1.1	Form of Underwriter Agreement**
2.1	Share Exchange Agreement dated as of June 8, 2006 among the Company, John S. Kovach and Lixte Biotechnology, Inc. 1
3.1	Certificate of Incorporation, as filed with the Delaware Secretary of State on May 24, 2005.
3.2	Certificate of Amendment of Certificate of Incorporation. ³
3.3	Certificate of Designations for the Company's Series A Convertible Preferred Stock. ⁴
3.4	Certificate of Amendment of Certificate of Designations of the Series A Convertible Preferred Stock. ⁵
3.5	Amended and Restated Bylaws ⁶
3.6	Certificate of Amendment of Certificate of Incorporation 7
3.7	Certificate of Amendment of Certificate of Incorporation ⁸
4.1	Form of Common Stock Certificate**
4.2	Certificate of Designations regarding the rights, preferences, privileges and restrictions with respect to any preferred stock**
4.3	Form of Preferred Stock Certificate**
4.4	Form of Debt Securities**
4.5	Form of Warrant**
4.6	Form of Warrant Agreement**
4.7	Form of Indenture*
4.8	Form of Rights Agreement (including Form of Rights, if any)**
4.9	Common Stock Purchase Warrant 9
5.1	Opinion of TroyGould PC***
10.1	Securities Purchase Agreement ¹⁰
23.1	Consent of Weinberg & Company, P.A., Independent Registered Public Accounting Firm*
23.2	Consent of TroyGould PC (included in Exhibit 5.1)
24.1	Power of Attorney (included on the signature page for the initial filing)
107	Filing Fee Table*

Filed as an Exhibit 2.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on July 7, 2006 and incorporated herein by reference.

- 2 Filed as an Exhibit 3.1 to the Company's Registration Statement on Form 10-SB, as filed with the Securities and Exchange Commission on August 3, 2005 and incorporated herein by reference.
- 3 Filed as Appendix A to the Company's Information Statement, as filed with the Securities and Exchange Commission on September 19, 2006 and incorporated herein by reference
- 4 Filed as an Exhibit 4.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on March 18, 2015 and incorporated herein by reference.
- 5 Filed as an Exhibit 3.4 to the Company's Annual Report on Form 10-K, as filed with the Securities and Exchange Commission on March 28, 2016 and incorporated herein by reference
- 6 Filed as an Exhibit 3.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on November 10, 2022 and incorporated herein by reference.
- 7 Filed as Exhibit 3.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on November 27, 2020.
- 8 Filed as Exhibit 3.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on June 6, 2023.
- 9 Filed as Exhibit 4.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on July 20, 2023.
- 10 Filed as Exhibit 10.1 to the Company's Current Report on Form 8-K, as filed with the Securities and Exchange Commission on July 20, 2023.
- * Previously Filed
- ** To be filed by amendment or as an exhibit to a document incorporated by reference in this registration statement, including a Current Report on Form 8-K in connection with the offering of any securities as appropriate
- *** Filed Herewith

TroyGould PC 1801 Century Park East, 16th Floor Los Angeles, California 90067

April 30, 2024

Lixte Biotechnology Holdings, Inc. 680 East Colorado Boulevard, Suite 180 Pasadena, California 91101

Re: Registration Statement on Form S-3

Ladies and Gentlemen:

We have acted as counsel to Lixte Biotechnology Holdings, Inc., a Delaware corporation (the "Company"), in connection with the above-referenced Registration Statement on Form S-3 (the "Registration Statement"), under the Securities Act of 1933, as amended (the "Securities Act"), filed by the Company with the Securities and Exchange Commission (the "Commission"). The Registration Statement relates to the Company's offer, sale, and issuance from time to time, pursuant to Rule 415 under the Securities Act, of the following securities of the Company with an aggregate public offering price of up to \$50,000,000: (1) shares of the Company's common stock, par value \$0.0001 per share ("Common Stock"); (2) shares of the Company's preferred stock, par value \$0.0001 per share ("Preferred Stock"), which may be issued in one or more series; (3) debt securities of the Company ("Debt Securities"), which may be issued in one or more series under an indenture proposed to be entered into by the Company and a trustee (the "Trustee") to be named in the indenture, the form of which is included as an exhibit to the Registration Statement, and one or more board resolutions, supplements thereto or officer's certificates thereunder (such indenture, together with the applicable board resolution, supplement or officer's certificate pertaining to the applicable series of Debt Securities, the "Applicable Indenture"); (4) warrants to purchase shares of Common Stock, shares of Preferred Stock, or Debt Securities, or any combination of such securities ("Warrants"); (5) stock purchase rights (the "Rights") entitling or obligating the holders thereof to purchase Common Stock or Preferred Stock from the Company at a future date or dates which will be issued pursuant to a rights agreement (the "Rights Agreement") to be entered into between the Company and a rights agent (the 'Rights Agent"); and (6) units consisting of any combination of shares of Common Stock, shares of Preferred Stock, Debt Securities, and/or Warrants (Units"). The Common Stock, the Preferred Stock, the Debt Securities, the Warrants, the Rights and the Units plus any additional Common Stock, Preferred Stock, Debt Securities, Warrants, Rights and Units that may be registered pursuant to any subsequent registration statement that the Company hereafter files with the Commission pursuant to Rule 462(b) under the Securities Act in connection with the offering contemplated by the Registration Statement collectively are referred to in this opinion letter as the "Securities". The Registration Statement also relates to an aggregate of 583,334 shares of Common Stock (the "Resale Shares") for offer and resale by the selling stockholder identified in the Registration Statement issuable by the Company upon exercise of currently outstanding common stock warrants (the "Selling Stockholder Warrants"). The Registration Statement includes two prospectuses (each, a "Prospectus"), which state that they will be supplemented from time to time by one or more supplements setting forth the specific terms of each offering of Securities (each, a "Prospectus Supplement").

This opinion letter is furnished to you at your request and in connection with the requirements of Item 601(b)(5) of Regulation S-K under the Securities Act.

In connection with rendering the opinions expressed below, we have reviewed and relied upon originals or copies of (1) the Registration Statement, (2) the Company's Certificate of Incorporation, as amended, including the Company's Certificate of Designations of Series A Preferred Stock, as presently in effect (the "Certificate of Incorporation"), (3) the Company's Amended and Restated Bylaws, as presently in effect (the "Bylaws"), (4) the resolutions adopted by the Company's Board of Directors (the "Board of Directors") pertaining to the Registration Statement, the Securities, and related matters, and (5) such certificates of public officials and officers of the Company, and such records of the Company and other documents, as we have deemed necessary or appropriate as a basis for our opinions. We also have reviewed such matters of the law described in the following paragraph as we considered necessary or appropriate as a basis for the opinions expressed below.

Lixte Biotechnology Holdings, Inc. April 30, 2024 Page 2

The law covered by our opinions expressed below is limited to (1) the General Corporation Law of the State of Delaware (the **Delaware Corporation Law**"), including applicable rules and regulations promulgated under the Delaware Corporation Law and applicable reported judicial decisions interpreting the Delaware Corporation Law, (2) the internal laws of the State of California, excluding laws, rules, and regulations of any counties, cities, municipalities, and local agencies within California, and (3) with respect only to the opinions expressed below in paragraph 3, 4 and 6 below regarding Debt Securities, Warrants and Units the internal laws of the State of New York, excluding laws, rules, and regulations of any counties, cities, municipalities, and local agencies within New York.

We neither express nor imply any opinion with respect to any other laws or the laws of any other jurisdiction. The Securities may be issued from time to time on a delayed or continuous basis, and the opinions expressed below concern only laws that are in effect on the date of this opinion letter. We undertake no, and hereby disclaim any, obligation to advise you of any change in any matter set forth in this opinion letter, whether based on a change in laws, a change in any fact relating to the Company, or any other circumstance. This opinion letter is limited to the matters expressly stated herein, and no opinions are to be inferred or may be implied beyond the opinions expressly set forth below. Without limiting the generality of the foregoing, we neither express nor imply any opinion regarding the contents of the Registration Statement, the Prospectus, or any Prospectus Supplement other than as expressly stated in this opinion letter with respect to the issuance of the Securities.

Based upon and subject to the foregoing and the additional assumptions, qualifications, and limitations set forth below, we are of the opinion that:

- 1. With respect to any shares of Common Stock offered by the Company pursuant to the Registration Statement, when (a) the issuance and the terms of the offering of such shares of Common Stock have been duly authorized and approved by all necessary corporate action by the Board of Directors in conformity with the Delaware Corporation Law and the Certificate of Incorporation, and (b) such shares have been duly issued and delivered against payment of their full purchase price in an amount not less than the par value of such shares and in accordance with the duly executed and delivered applicable purchase, underwriting, or similar agreement approved by the Board of Directors and the terms of the Registration Statement, the Prospectus, and the applicable Prospectus Supplement (and, if issued upon the exercise, conversion, or exchange of any Securities that are exercisable for, convertible into, or exchangeable for Common Stock, when such shares of Common Stock have been duly issued and delivered as contemplated by the exercise, conversion, or exchange terms of such Securities). In rendering the foregoing opinion, we have assumed that the Company will comply with all applicable notice requirements regarding uncertificated shares provided in the Delaware Corporation Law.
- 2. With respect to any shares of any series of Preferred Stock offered by the Company pursuant to the Registration Statement, when (a) the issuance and the terms of the offering of such shares of Preferred Stock have been duly authorized and approved by all necessary corporate action by the Board of Directors in conformity with the Delaware Corporation Law and the Certificate of Incorporation, including a duly adopted certificate of designations setting forth the rights, preferences, and privileges of such series of Preferred Stock conforming to the Delaware Corporation Law and filed with the Secretary of State of the State of Delaware, and (b) such shares have been duly issued and delivered against payment of their full purchase price in an amount not less than the par value of such shares and in accordance with the duly executed and delivered applicable purchase, underwriting, or similar agreement approved by the Board of Directors and the terms of the Registration Statement, the Prospectus, and the applicable Prospectus Supplement (and, if issued upon the exercise, conversion, or exchange of any Securities that are exercisable for, convertible into, or exchangeable for Preferred

Stock, when such shares of Preferred Stock have been duly issued and delivered as contemplated by the exercise, conversion, or exchange terms of such Securities), such shares of Preferred Stock will be validly issued, fully paid, and nonassessable. In rendering the foregoing opinion, we have assumed that the Company will comply with all applicable notice requirements regarding uncertificated shares provided in the Delaware Corporation Law.

Lixte Biotechnology Holdings, Inc. April 30, 2024 Page 3

- 3. With respect to any series of Debt Securities offered by the Company pursuant to the Registration Statement, when (a) the Applicable Indenture has been duly qualified under the Trust Indenture Act of 1939, as amended (the "Trust Indenture Act"), (b) the Trustee has been duly qualified to act as trustee under the Applicable Indenture following the filing of a Form T-1 with the Commission, (c) the issuance and the terms of the offering of such Debt Securities have been duly authorized and approved by all necessary corporate action by the Board of Directors, (d) the Applicable Indenture has been duly executed and delivered by the Company following authorization and approval by all necessary corporate action by the Board of Directors, (e) the Applicable Indenture has been duly executed and delivered by the Trustee, (f) such Debt Securities have been duly executed, authenticated, issued, and delivered against payment of their full purchase price and in accordance with the Indenture, the duly executed and delivered applicable purchase, underwriting, or similar agreement approved by the Board of Directors, and the terms of the Registration Statement, the Prospectus, and the applicable Prospectus Supplement (and, if issued upon the exercise, conversion, or exchange of any Securities that are exercisable for, convertible into, or exchangeable for Debt Securities, when such Debt Securities have been duly issued and delivered as contemplated by the exercise, conversion, or exchange terms of such Securities), and (g) any Securities that are issuable upon conversion of such Debt Securities have been duly authorized and approved (and, if applicable, reserved for issuance) by all necessary corporate action by the Board of Directors, such Debt Securities will constitute valid and binding obligations of the Company, enforceable against the Company in accordance with their terms.
- 4. With respect to any Warrants offered by the Company pursuant to the Registration Statement, when (a) the issuance and the terms of the offering of such Warrants have been duly authorized and approved by all necessary corporate action by the Board of Directors, (b) such Warrants have been duly executed, issued, and delivered against payment of their full purchase price and in accordance with the duly executed and delivered applicable warrant, purchase, underwriting, or similar agreement approved by the Board of Directors and the terms of the Registration Statement, the Prospectus, and the applicable Prospectus Supplement, and (c) the Securities that are issuable upon exercise of such Warrants have been duly authorized and approved (and, if applicable, reserved for issuance) by all necessary corporate action by the Board of Directors, such Warrants will constitute valid and binding obligations of the Company, enforceable against the Company in accordance with their terms.
- 5. With respect to any Rights offered by the Company pursuant to the Registration Statement, when (a) the issuance and terms of the offering of such Rights have been duly authorized and approved by all necessary corporate action by the Board of Directors. (b) a Rights Agreement relating to such Rights has been duly authorized, executed and delivered by the Company and duly executed by the Rights Agent named in the Rights Agreement; (c) if such Rights relate to the issuance and sale of Common Stock, the actions described in paragraph 1 above have been taken; (d) if such Rights relate to the issuance and sale of Preferred Stock, the actions described in paragraph 2 above have been taken, and (e) certificates representing such Rights have been duly executed, countersigned and registered in accordance with the Rights Agreement and have been duly delivered in accordance with the Rights Agreement against payment of the agreed consideration therefor, such Rights will constitute valid and binding obligations of the Company in accordance with their terms.
- 6. With respect to any Units offered by the Company pursuant to the Registration Statement, when (a) the issuance and the terms of the offering of such Units have been duly authorized and approved by all necessary corporate action by the Board of Directors, (b) such Units have been duly issued and delivered (and, if such Units are represented by certificates, such certificates representing Units have been duly executed and delivered) against payment of their full purchase price and in accordance with the duly executed and delivered applicable unit, purchase, underwriting, or similar agreement approved by the Board of Directors and the terms of the Registration Statement, the Prospectus, and the applicable Prospectus Supplement, and (c) the Securities that are issuable as part of or upon exercise of such Units have been duly authorized and approved (and, if applicable, reserved for issuance) by all necessary corporate action by the Board of Directors, such Units will constitute valid and binding obligations of the Company, enforceable against the Company in accordance with their terms.
- 7. With respect to any Resale Shares offered by the Company pursuant to the Registration Statement, the Resale Shares, when issued and delivered by the Company upon exercise of the Selling Stockholder Warrants in accordance with the terms thereof, will be validly issued, fully paid, and non-assessable.

Lixte Biotechnology Holdings, Inc. April 30, 2024 Page 4

With your permission, we have made (and are relying upon) the following assumptions, without any independent investigation or inquiry by us, and our opinions expressed above are subject to, and limited and qualified by the effect of, the following assumptions: (1) all representations, warranties, and other statements as to factual matters that are contained in the documents that we reviewed in connection with this opinion letter are accurate and complete, and all corporate records furnished to us by the Company are accurate and complete; (2) the Registration Statement will be declared effective under the Securities Act prior to the Company's offer, sale, or issuance of any Securities, and such effectiveness will not be suspended or terminated as of the date of the offer, sale, or issuance of any Securities; (3) the Board of Directors will adopt resolutions duly authorizing each offer, sale, and issuance of the Securities and establishing the terms of the offering of such Securities, and such resolutions will not be rescinded and will not be modified in a manner that adversely affects the opinions expressed above; (4) in compliance with the Securities Act and the rules and regulations thereunder, the Company will prepare and file with the Commission a Prospectus Supplement describing the terms of each offering of any Securities in compliance with the Securities Act and the rules and regulations thereunder; (5) a definitive purchase, underwriting, warrant, rights agreement, unit, or similar agreement will be duly executed and delivered by the Company and the other parties thereto with respect to each offer, sale, and issuance of any Securities, and each such agreement will constitute the valid and binding obligation of each party other than the Company enforceable against each party other than the Company in accordance with its terms; (6) if Debt Securities are issued, the Applicable Indenture will be duly executed and delivered by the Trustee and will constitute the valid and binding obligation of the Trustee, enforceable against the Trustee in accordance with its terms, and the executed Applicable Indenture will not differ in any material respect from the form of Applicable Indenture that is filed as an exhibit to the Registration Statement; (7) if Rights are issued, a Rights Agreement will be duly executed and delivered by the Rights Agent and will constitute the valid and binding obligation of the Rights Agent, enforceable against the Rights Agent in accordance with its terms; (8) the number of shares of Common Stock or Preferred Stock, as applicable, to be issued pursuant to the Registration Statement, the Prospectus, and any Prospectus Supplement, together with the number of then-outstanding shares of Common Stock or Preferred Stock, will not exceed the number of shares of Common Stock or Preferred Stock authorized in the Certificate of Incorporation; (9) in connection with each issuance of any Securities, the Company will duly execute and deliver stock certificates, promissory notes, warrant certificates, or unit certificates, as applicable, in the form filed, or to be filed, by the Company as exhibits to the Registration Statement or in the form of the applicable documents to be filed by the Company with the Commission and incorporated by reference into the Registration Statement, provided, that, with respect to any Common Stock, Preferred Stock, or Units issued on an uncertificated basis, the Company will comply with applicable law regarding notice requirements and the documentation of uncertificated securities; (10) all Securities will be offered, sold, and issued by the Company in compliance with applicable federal and state securities laws, rules, and regulations, including, without limitation, the Securities Act and the Trust Indenture Act, and the rules and regulations thereunder, and in the manner stated in the Registration Statement, the Prospectus, and the applicable Prospectus Supplement; (11) the Company's offer, sale, and issuance of the Securities, and compliance with any definitive purchase, underwriting, warrant, rights agreement, unit, or similar agreement, or with the Applicable Indenture, pertaining to such offer, sale, and issuance, will not constitute a default under, or a breach of, any agreement to which the Company is a party or is otherwise subject, and neither the Certificate of Incorporation nor the Bylaws will be amended after the date of this opinion letter in a manner that would cause such offer or sale

of any Securities to constitute a violation of the Certificate of Incorporation or Bylaws; (12) each purchase, underwriting, warrant, rights agreement unit, or similar agreement pertaining to the offer, sale, and issuance of any Securities will be governed by the internal laws of the State of California, except that the Applicable Indenture, and any related promissory notes will be governed by the internal laws of the State of New York; (13) if any Securities are issued by the Company upon the exercise, conversion, or exchange of other Securities, the exercise, conversion, or exchange terms of such Securities, as applicable, will be complied with; and (14) with respect to documents that we reviewed in connection with this opinion letter, all documents submitted to us as originals are authentic and complete; all documents submitted to us as certified, electronic, facsimile, or photostatic copies conform to the originals of such documents, and such original documents are authentic and complete; the signatures on all documents are genuine; and all natural persons who have executed any of the documents have the legal capacity to do so.

Lixte Biotechnology Holdings, Inc. April 30, 2024 Page 5

Our opinions are subject to (a) the effect of bankruptcy, insolvency, reorganization, preference, fraudulent transfer, moratorium, and other similar laws and court decisions relating to or affecting the rights and remedies of creditors, (b) the effect of general principles of equity, whether considered in a proceeding in equity or at law (including the possible unavailability of specific performance or injunctive relief), concepts of materiality, reasonableness, good faith, and fair dealing, and the discretion of the court before which a proceeding is brought, and (c) the invalidity under certain circumstances under law or court decisions of provisions providing for the indemnification of or contribution to a party with respect to a liability where such indemnification or contribution is contrary to public policy or otherwise illegal. Furthermore, we neither express nor imply any opinion as to (1) any provision for liquidated damages, default interest, default charges, late charges, monetary penalties, make-whole premiums, or other economic remedies to the extent such provisions are deemed to constitute a penalty, (2) consents to, or restrictions upon, governing law, jurisdiction, venue, arbitration, remedies, or judicial relief, (3) waivers of rights or defenses, (4) any provision requiring the payment of attorneys' fees, where such payment is contrary to law or public policy, (5) any provision permitting, upon acceleration of any Debt Securities, collection of that portion of the stated principal amount thereof which might be determined to constitute unearned interest thereon, (6) the creation, validity, attachment, perfection, or priority of any lien or security interest, (7) advance waivers of claims, defenses, rights granted by law, notice, opportunity for hearing, evidentiary requirements, statutes of limitation, trial by jury or at law, or other procedural rights, (8) waivers of broadly or vaguely stated rights, (9) provisions for exclusivity, election, or cumulation of rights or remedies, (10) provisions authorizing or validating conclusive or discretionary determinations, (11) grants of setoff rights, (12) proxies, powers, and trusts, (13) any law, rule, or regulation relating to usury, (14) any provision to the extent it requires that a claim with respect to a security denominated in other than U.S. dollars (or a judgment in respect of such a claim) be converted into U.S. dollars at a rate of exchange at a particular date, to the extent applicable law otherwise provides, or (15) the severability, if invalid, of provisions to the foregoing effect. We neither express nor imply any opinion regarding the validity, binding effect, or enforceability of any agreement except to the extent expressly stated above in this opinion letter.

This opinion letter is rendered to you in connection with the Registration Statement and may not be relied upon by you for any other purpose. We hereby consent to the filing of this opinion letter as an exhibit to the Registration Statement and further consent to the reference to our name under the caption "Legal Matters" in the Prospectus. We further consent to the incorporation by reference of this letter and consent into any registration statement or post-effective amendment to the Registration Statement filed pursuant to Rule 462(b) under the Act with respect to the Securities. In giving our consent, we do not thereby admit that we are included within the category of persons whose consent is required under Section 7 of the Securities Act or the rules and regulations of the Commission under the Securities Act.

Very truly yours,

/s/ TroyGould PC

TROYGOULD PC